ENDOCRINOLOGY

VOLUME 34 APRIL, 1944 NUMBER 4

THE RELATION OF AGE TO REACTIVITY IN THE REPRODUCTIVE SYSTEM OF THE RAT[1,2]

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INTRODUCTION

THE WORK of Smith (1926, 1927) and Smith and Engle (1927a, b) brought out the fact that in male and female rats and mice below weaning age more pituitary implants were necessary to produce precocious development of the reproductive system than in older rats. This suggested that the gonads might be unable to give a maximal response to such stimulation until they had reached a certain degree of development or maturity. Since that time, many studies have been made on the response of embryonic, immature and mature gonads and accessory reproductive organs to gonadotropic and sex hormones. It is definitely established that age and sex of the animal are related to the degree of reactivity to hormone stimulation as determined by changes in organ weights and histology and in behavior responses. However, it is difficult to gain a clear understanding from the accumulated data of the process of development of reactivity in the various parts of the male and female reproductive tract in relation to age because of the lack of uniformity in hormone dosage, length of administration and methods of judging effects.

Received for publication November 9, 1943.

[1] A portion of this work on organ weights was submitted as a master's thesis by Evelina Ortiz and is on file in the University of Chicago Libraries. The problem developed from suggestions made by Dr. Carl R. Moore.

[2] This investigation has been aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund. We are indebted to Dr. Gregory Stragnell of the Schering Corporation for testosterone propionate (Oreton) and estradiol benzoate (Progynon-B) used in this work, to Dr. Donald Wonder, Cutter Laboratories for equine gonadotropin (Gonadin) and to Mr. Kenji Toda who prepared the graph and photomicrographs. The Biology Department of the University of Puerto Rico kindly provided technical facilities for the preparation of some of the slides.

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For this reason it seemed advisable to study the problem, using a standardized method with the same hormone dosages administered for the same period of time at different ages from birth to maturity, and judging responsiveness by changes in weight and in histology of the organs. The stimulation or inhibition of hormone production in the gonads was determined by study of the changes in the accessory reproductive glands.

MATERIAL AND METHODS

The rats used in these experiments were from a stock maintained in this laboratory for over 20 years. In some of the work, animals from an inbred stock were employed to study effects on the female prostate, which occurs spontaneously in only a small percentage of the females in our common laboratory stock. A total of 1077 rats was used and of these 720 were injected and 357 served as controls. In all experiments, the litter was used as a unit.

Series of experiments were performed, using equine gonadotropin, testosterone propionate and estradiol benzoate. These hormones were injected subcutaneously in daily dosages of 10 R.U. (20 I.U.) of equine gonadotropin, 0.1 mg. of testosterone propionate and 1 R.U. of estradiol benzoate. The gonadotropin was in aqueous solution, the sex hormones were dissolved in sesame oil, and daily doses were given in 0.1 cc. of medium. Injections were made for a period of 6 days beginning on the day of birth, 4, 8, 12, 20, 30 or 50 days of age. The animals were autopsied the day after the last injection at 6, 10, 14, 18, 26, 36 or 56 days of age. A binocular dissecting microscope was used and organs were weighed on a torsion balance immediately after dissection. The seminal vesicles and coagulating glands were weighed together at 6 days of age because of their small size but they were weighed separately at all subsequent ages. It was difficult to separate the ovary from the oviduct in the youngest animals and as routine the two were weighed together at all ages in the first series of experiments. This was unsatisfactory, and in later series, the ovaries and oviducts were weighed separately at all ages except 6 days. The number of animals in which separate weights were taken is shown in parentheses in table 1 to the right of the figure representing the total number used. Since the horns of the uteri were to be fixed for sectioning after they were weighed, no attempt was made to remove the fluid which accumulated in the uteri of older animals which were injected with gonadotropin or estradiol benzoate. Injection of testosterone propionate did not result in stimulation of functional activity of the uterine endometrium and the uterine weights were not increased by additional fluid.

Weights of gonads and accessory reproductive glands of injected animals were compared with those of untreated littermate controls, the average weights were computed and the percentage increase or decrease determined for each litter. The average of these percentages was then obtained for all litters similarly treated. These data are given in table 1, and shown in figure 17. Table 2 presents data from litters which demonstrate some of the effects on organ weights. Males and females listed in table 2 are from different litters. Data for the coagulating gland were omitted from table 2 and the graph since it followed essentially the same curve as the seminal vesicle. No effects of the injected hormones on body weights were found.

The organs were fixed in Bouin's fluid, sectioned, and stained in Harris'

or Ehrlich's hematoxylin. Ovaries, female prostate glands and parts of the uteri were cut serially at 10μ, and samples of the testes and male accessories were sectioned at 7μ. Tissues from 248 animals were studied. No histological study was made of oviducts or coagulating glands.

To aid in accurate comparison of the organs, ocular micrometer measurements were made of the diameters of testis tubules and ovarian follicles. In some animals, follicles were counted in cross sections of the ovaries.

Observations on the size of the penis and clitoris were made during the course of the experiments and at autopsy. Any effects on vaginal opening were noted and vaginal smears were made in some cases.

The reaction of the female prostate gland in relation to age constitutes a separate study and will be reported elsewhere.

EXPERIMENTAL RESULTS

Equine gonadotropin injections.

Autopsy at 6 days of age.

The injection of 10 R.U. of gonadotropin daily into male rats for 6 days produced an average increase of 48 per cent in testis weight. Weight increases were found in all injected animals. A typical example of the changes in gross weight of the testes is shown in litter 1, table 2. Histological study brought out that in the testes of injected males there were large localized groups of cells resembling interstitial cells, also, the tubules were somewhat separated, indicating possible edema, but there seemed to be no increase in tubular diameter or change in the germinal epithelium (compare figs. 1 and 2). There was some evidence of increased hormone production in the testes of the experimental animals in that the combined seminal vesicle and coagulating gland weights were 25 per cent heavier than those of controls. An increase was not found in all treated males, but in some litters (as in litter 1, table 2) weight increases were consistent. Prostate weight was unaffected, and neither the prostate nor the seminal vesicle of the 6-day-old treated males was stimulated histologically.

The 6-day-old injected females were less responsive to gonadotropic treatment than the males. The ovaries and oviducts showed weight increases of only 7 per cent on the average, the uteri none at all (see table 1, and litter 1, table 2), and no histological changes were found.

Autopsy at 10 days of age.

At this age the males showed greater reactivity to gonadotropin than at 6 days. There was an average increase of 84 per cent in testis weight. Interstitial cells were scarce in the control testes but numerous in the testes of treated males. These cells were still in groups, although the groups were not so large as at 6 days. The tubules were solid and diameters were approximately the same as those in controls, but a slight difference was detectable in the germinal epithelium, which seemed less crowded with nuclei than in the testis tubules of controls. Since mitoses were strikingly numerous in the germinal epithelium

of control and injected animals it is possible that the appearance of the testis tubules of experimental males indicated an increase in tubular length, which might account for part of the increase in testis weight.

The prostate was definitely stimulated by the increased amount of male hormone from the testes and responded with a 16 per cent

TABLE 1. SUMMARY OF RESPONSE OF ORGANS IN AVERAGE PERCENTAGE CHANGE IN WEIGHT

Treatment	Aut. Age in Days	No. of Rats	Control		Treated		Ov.	Ovd.	Ov. + Ovd.	Ut.	Test.	V.pr.	S.V.	C.G.
			♀	♂	♀	♂								
Equine gonadotropin. 10 R.U. daily for 6 days	6	44	8	6	15	15			7	−.06	48	2	25	
	10	38	7(3)	3	19(4)	9	60	36	54	82	84	16	118	51
	14	40	6(2)	4	20(4)	10	173	97	125	265	125	139	258	186
	18	46	9(4)	5	20(5)	12	254	86	178	561	90	205	350	284
	26	26	6(6)	4	10(10)	6	2928	239	2305	675	99	120	656	438
	36	31	6(3)	4	12(4)	9	1540	97	1395	538	15	149	545	421
	56	53	9(5)	8	20(9)	16	543	29	309	121	− 3	59	165	121
Total		278	51	34	116	77								
Testosterone propionate .1 mg. daily for 6 days	6	43	5	7	18	13			74	25	−21	5	52	
	10	62	7(2)	12	15(3)	28	− 4	15	16	48	−15	40	124	104
	14	88	18(11)	11	36(13)	23	− 6	42	28	75	− 2	114	279	226
	18	74	13(7)	14	27(13)	20	−27	50	13	114	−0.05	104	494	430
	26	34	9(9)	5	12(12)	8	2	86	23	375	−16	113	742	390
	36	58	12(7)	9	21(8)	16	0.5	46	17	344	−30	81	575	334
	56	56	13(5)	5	24(7)	14	−13	8	−6	41	−13	30	54	44
Total		415	77	63	153	122								
Estradiol benzoate 1 R.U. daily for 6 days	6	35	8	6	11	10			55	171	− 4	−13	−22	
	10	35	7(3)	4	16(3)	8	−16	87	27	235	−17	−25	−24	−50
	14	57	15(9)	4	25(11)	13	−18	47	16	258	−16	−15	−20	14
	18	61	14(8)	6	27(10)	14	−17	66	14	356	−14	−18	−13	−14
	26	44	12(12)	6	15(15)	11	18	105	37	440	−27	−24	−13	−42
	36	77	15(9)	11	27(11)	24	40	70	39	365	− 9	− 9	−22	−19
	56	75	16(5)	8	33(7)	18	− 8	−0.2	−2	31	− 6	− 2	9	− 5
Total		384	87	45	154	98								
Grand Total		1077	215	142	423	297								

Ov. = ovary; Ovd. = oviduct; Ut. = uterus; Test. = testis; V.pr. = ventral prostate; S.V. = seminal vesicle; C.G. = coagulating gland.

weight increase. In the injected males the gland had greater acinous distention and moderately high epithelium in which could be seen the light areas typical of a normal functioning gland. These light areas do not normally appear until about 12 days of age.

The seminal vesicles were increased 118 per cent in weight and had a moderately complicated structure as compared with the simple lumen and side branches of the control glands, but in both control and injected animals the seminal vesicle epithelium presented the pseudo-stratified appearance characteristic of the undeveloped gland.

The females of this age also showed greater reactivity to treatment than at 6 days. The ovaries were increased 60 per cent in weight but differed histologically from the controls only in respect to the theca, which was increased in width in the experimental animals. Mitoses were far more numerous in the theca of the injected rats and were also seen in abundance in the granulosa.

The uteri of injected females were increased 82 per cent in weight.

The epithelium was increased in height and the lumen, in addition to being larger, had a few more mucosal folds in some cases. Glands were beginning to develop in the uteri of both control and experimental animals and were seemingly not stimulated to greater development by the increased female hormone. In fact, the glands were somewhat more conspicuous in the control uteri. The clitoris was not enlarged.

Autopsy at 14 days of age.

There was an increase in testis weight of 125 per cent in injected males at 14 days autopsy age. As at younger ages, tubular diameters were not increased nor was the germinal epithelium stimulated. Interstitial cells were markedly increased, as before, and were in small groups. Some evidence of precocious development was found in that small lumina were beginning to form in the testis tubules of the injected males. Lumina normally develop in the tubules between 15 and 18 days of age. The testis tubules were somewhat dispersed as at younger ages.

The prostate responded to gonadotropin stimulation by increasing 139 per cent in weight and was much better developed histologically than the control glands. The seminal vesicles averaged 258 per cent heavier than those of controls and were more complicated in structure, with moderately high epithelium in which there were small secretion granules which do not normally appear until about 36 days of age.

At this age the penis of treated males was enlarged and the testes had descended into the scrotum. Testis descent normally occurs in rats of our colony at about 25 days of age.

The ovaries of injected females at 14 days autopsy age were 173 per cent heavier than those of controls and were definitely advanced in histological development. The largest follicles were larger than those in control ovaries, the antra were larger, and there were more follicles with antra. There was greater increase in thecal development than in the ovaries of treated females at 10 days autopsy age.

The uterine weight of treated females was increased 265 per cent. Stimulation was evident histologically. The lumina contained more mucosal folds and the epithelium was higher than in the uteri of controls. Glands were less conspicuous. The clitoris of treated females was larger than that of controls.

Autopsy at 18 days of age.

In treated males at this age, the testes were 90 per cent heavier than those of controls and were advanced in histological development. The tubules were slightly increased in diameter and lumina were larger than in the testis tubules of controls, but spermatogenesis was unaffected. As in all previous ages, the tubules in the injected animals were somewhat separated and groups of interstitial cells were abundant. Litter 2 in table 2 shows the absolute weight increases.

Prostate glands of injected males were increased 205 per cent in

weight and showed stimulation histologically. Seminal vesicles were increased 350 per cent and were further developed histologically than in the 14-day-old injected males and were far more advanced than the glands of controls. The penes were enlarged and the testes had descended into the scrotum.

Ovaries responded at 18 days by an average increase of 254 per cent in weight (see litter 2, table 2). Decided histological changes were found, all of which, however, had begun to be evident at younger ages. Follicles were larger and the theca and interstitial tissue were increased. The theca appeared luteinized. Comparison of figures 8 and

TABLE 2. EFFECT OF HORMONES ON ORGAN WEIGHTS

Females							Males				
No.	Aut. Age in Days	Treat.[1]	Body Wt.	Ov.	Ovd.	Ut.	Treat.[1]	Body Wt.	Test.	V.pr.	S.V.[2]
			g.	*mg.*	*mg.*	*mg.*		*g.*	*mg.*	*mg.*	*mg.*
1	6	Eq. Gon.	9	1.2		3.4	Eq. Gon.	10	25.0	1.8	2.4
		Eq. Gon.	9	1.6		4.6	Eq. Gon.	10	23.8	1.6	2.6
		Eq. Gon.	8	1.8		3.8	Eq. Gon.	10	22.8	2.0	2.8
		Eq. Gon.	8	2.4		4.6	Control	10	14.2	1.2	1.6
		Control	9	1.8		4.2	Control	10	13.8	1.6	1.6
2	18	Eq. Gon.	21	22.8	6.2	94.4	Eq. Gon.	24	194.2	16.6	18.2
		Eq. Gon.	21	20.0	6.6	82.4	Eq. Gon.	26	210.0	20.0	19.6
		Eq. Gon.	21	23.0	7.0	96.8	Eq. Gon.	26	234.6	21.6	23.2
		Control	25	8.8	4.8	25.0	Control	24	98.8	8.6	4.0
		Control	21	5.6	3.6	17.8	Control	22	103.0	11.0	5.0
3	26	Eq. Gon.	39	519.0	14.2	149.0	Eq. Gon.	47	581.0	59.0	44.8
		Eq. Gon.	34	412.4	16.2	153.6	Eq. Gon.	46	725.6	62.2	53.8
		Eq. Gon.	35	462.0	12.6	167.2	Control	48	343.8	26.4	6.8
		Control	39	14.6	4.0	26.4					
		Control	35	15.8	4.6	23.4					
4	56	Eq. Gon.	104	206.0	16.6	351.8	Eq. Gon.	103	1690.0	180.6	403.6
		Eq. Gon.	92	175.6	17.0	277.6	Eq. Gon.	94	1730.0	184.8	448.4
		Eq. Gon.	89	177.2	16.8	245.0	Eq. Gon.	99	1570.0	172.2	330.8
		Control	95	25.6	16.2	97.8	Control	130	1990.0	132.2	232.0
							Control	97	1760.0	79.6	86.2
5	6	T. prop.	11	3.6		5.0	T. prop.	14	16.4	2.0	3.6
		T. prop.	11	3.6		5.4	T. prop.	14	16.2	2.0	4.2
		T. prop.	12	3.8		5.4	T. prop.	13	13.8	1.6	2.8
		Control	11	1.8		3.6	Control	13	18.4	1.8	1.8
		Control	11	2.1		4.1	Control	14	18.6	1.6	2.4
6	18	T. prop.	22	4.6	6.2	34.0	T. prop.	23	89.8	14.2	22.0
		T. prop.	23	5.2	6.2	44.0	T. prop.	22	88.2	14.2	20.6
		Control	23	6.2	4.4	19.8	Control	21	83.8	6.6	4.0
7	36	T. prop.	65	18.6	8.6	93.0	T. prop.	82	590.0	104.2	91.4
		T. prop.	79	22.0	10.4	109.6	T. prop.	79	562.8	95.6	87.6
		Control	69	16.6	6.4	36.0	T. prop.	84	609.2	100.0	81.4
		Control	83	20.0	8.2	27.0	Control	79	850.2	58.2	22.8
							Control	85	876.0	54.6	27.8
8	6	Estr. B.	9	2.4		9.8	Estr. B.	13	14.6	1.0	1.6
		Estr. B.	9	3.2		9.2	Estr. B.	10	12.6	0.6	1.0
		Estr. B.	9	2.6		7.8	Estr. B.	11	14.8	1.0	1.0
		Estr. B.	9	2.8		8.6	Estr. B.	10	12.0	0.6	1.0
		Control	8	1.8		3.4	Control	12	16.6	1.2	1.2
							Control	9	11.8	0.8	1.6
9	18	Estr. B.	28	8.2	8.4	95.6	Estr. B.	23	84.6	6.4	4.0
		Estr. B.	27	7.0	7.0	81.8	Estr. B.	25	83.4	7.2	4.0
		Control	25	9.6	4.8	20.0	Control	26	103.2	9.4	5.0
		Control	25	8.6	5.0	19.0					
10	36	Estr. B.	72	24.8	8.6	123.8	Estr. B.	82	773.8	51.0	17.4
		Estr. B.	66	23.8	11.8	132.6	Estr. B.	94	991.0	64.0	23.8
		Control	68	15.8	6.4	25.8	Control	89	1338.0	66.8	28.4

[1] Daily doses of 10 R.U. of equine gonadotropin, 0.1 mg. testosterone propionate, and 1 R.U. of estradiol benzoate for 6 days; autopsy 24 hours after last injection.
[2] Seminal vesicles and coagulating glands were weighed together at 6 days.

10 with figures 11 and 12 brings out the degree of ovarian stimulation.

The increased female hormone from such stimulated ovaries produced weight increases of 561 per cent in the uteri, and marked histological differentiation (compare figs. 7 and 9). Glands appeared to be more numerous and conspicuous in the control uteri, but they were found also in the uteri of experimental animals and were larger than those in controls. Fluid was present in the uteri of injected females.

The clitoris was enlarged by gonadin treatment, and in one litter the vagina opened in all of the treated females. Vaginal opening normally occurs at approximately 50 days in our stock of rats.

Autopsy at 26 days of age.

Testes of males at 26 days were increased 99 per cent in weight by gonadotropin injection and contained more interstitial cells. Tubular diameters were possibly slightly increased but no evidence of advanced spermatogenesis was found. The prostate was increased 120 per cent in weight and the seminal vesicles 656 per cent (see litter 3, table 2). The prostate of experimental animals was not advanced histologically except for a slight increase in acinous distention but the seminal vesicle was greatly stimulated and had a high epithelium containing secretion granules.

In treated females autopsied at 26 days of age the ovaries had developed great reactivity and were increased 2928 per cent (see litter 3, table 2). The average gross weight of the treated ovaries was 450 mg. as compared with 18 mg., the average for the control ovaries. Histologically, the ovaries of 26-day-old injected females contained many large follicles, conspicuous blood points and a few corpora lutea.

The uteri of injected females were 675 per cent heavier than control uteri and were distended with fluid; many mucosal folds projected into the lumina; the tall columnar uterine epithelium was vacuolated, and glands were large and had a high epithelium. Control uteri were essentially as undeveloped as at 18 days (fig. 7). Vaginal opening had occurred in all treated females and the smears indicated estrus or proestrus.

Autopsy at 36 and 56 days of age.

In injected males autopsied at 36 days an average increase of 15 per cent was obtained in testis weights. Interstitial cells were markedly increased and were dispersed evenly between the tubules. Tubular diameters were unchanged and metamorphosing spermatozoa were as numerous in the controls as in the injected males. The prostate weight was increased 149 per cent, and the seminal vesicle, 545 per cent. Histologically, the prostate glands of treated males showed no stimulation, but the seminal vesicles had much higher epithelium and larger secretion granules than their controls. At 56 days the testes were not consistently increased in weight and did not differ histologically from control testes except for the increased numbers of interstitial cells. Mature sperm were present in the testis tubules of both

control and experimental males. The prostates of injected males were 59 per cent heavier than those of controls, and the seminal vesicles 165 per cent. Prostates and seminal vesicles did not differ histologically from control glands. The penis of injected males was enlarged at 36 days and slightly so at 56 days.

The average weight of the ovaries of 36-day-old injected females was increased 1540 per cent. The average gross weight was 375 mg. There were marked changes in the size of the follicles and many corpora lutea were formed (compare figs. 13 and 14). No corpora lutea were found in the ovaries of control females.

Uteri of injected females were 538 per cent heavier than control uteri, and were greatly stimulated histologically, as at 26 days. Vaginal opening occurred in 9 of the 12 females and estrous smears were obtained. The vagina remained closed in all controls. The clitoris was enlarged in all treated females.

The injection of equine gonadotropin into females killed at 56 days enlarged the ovaries 543 per cent but their average weight was only 175 mg. Litter 4 in table 2 serves as an example of the absolute weight increases. The histological changes in the experimental ovary are illustrated in figure 16 and the normal control ovary is shown in figure 15. The loss in reactivity to stimulation of the ovary at 56 days is striking when one compares figure 16 with figure 13, the ovary of a 36-day-old treated female.

The uterine weights of injected females were 121 per cent heavier than those of the controls, which at this age (see litter 4) had shown a great normal increase. Histologically, the uteri of injected females were markedly stimulated. The vagina had opened in controls as well as in injected females at 56 days. Smears indicated that both groups were beginning cycles.

In summary it may be stated that the gonads of rats were responsive to gonadotropic treatments very early (testes between birth and 6 days; ovaries between 4 and 10 days), but their first response was relatively small. Reactivity increased rapidly with age and reached a peak at 14 days in the testis and 26 days in the ovary, after which ability to increase in weight above the normal declined. Gonad responsiveness was shown by weight increase, histological changes and increased hormone production which affected the accessory organs as early as 6 and 10 days. These organs, in turn, showed increased reactivity with increasing age and reached a maximum weight response in the prostate at 18 days of age, and at 26 days in the other accessory organs. By 56 days reactivity appeared greatly reduced.

Testosterone propionate injections.

Autopsy at 6 days of age.

The injection of 0.1 mg. daily of testosterone propionate into young males from birth to 6 days of age produced a decrease of 21

per cent in testis weight. Litter 5 in table 2 serves to illustrate the range in weight loss. No detectable histological differences could be found, however. The weight of the prostate gland was not significantly increased by direct stimulation with male hormone although, as in litter 5, the weights were larger in some injected animals. Histologically, there was some indication of stimulation, for small lumina had formed in many of the acini, whereas in the control glands all acini were solid and small. The combined weights of seminal vesicles and coagulating glands were increased 52 per cent. These increases were comparatively small but consistent. The seminal vesicles were larger in cross section than those of the controls but did not show other histological evidence of stimulation. The penis of injected males was enlarged.

The combined weights of ovaries and oviducts were increased 74 per cent. It is probable that this represents an increase in the oviduct weight which forms a large part of the combined weights at this age. The uterus was stimulated in growth and weighed 25 per cent more than the uteri of controls (see litter 5) but there was no specific histological change. The clitoris was enlarged in all injected females.

Autopsy at 10 days of age.

At this age the testes were reduced 15 per cent in weight but, as at 6 days, no histological change was found, with the possible exception that mitoses seemed more numerous in the control testes. The prostate weighed 40 per cent more than that of controls and was advanced in differentiation as shown in figure 6. The acini were larger than those of the control (fig. 5); the epithelium was higher and precociously developed light areas were present in the cells. The seminal vesicles were 124 per cent heavier than those of controls and were stimulated histologically (fig. 4). The structure was more complicated than that of the control (fig. 3) and the cytoplasm contained small secretion granules, which are not found in seminal vesicles of untreated rats until approximately 36 days of age. Penes of males were enlarged by the treatment.

The ovaries of 10-day-old injected females were not significantly reduced in weight and no histological change was found. The uteri were increased 48 per cent in weight but were indistinguishable from those of controls except in size. The clitoris was enlarged as at 6 days.

Autopsy at 14 days of age.

Testes of treated males killed at 14 days were not affected in weight or in histology. The prostate glands were 114 per cent heavier than those of controls and were advanced histologically. The seminal vesicles were increased 279 per cent in weight and stimulation was evident histologically. Secretion granules were better developed than in the seminal vesicles of injected males at 10 days. The penis was enlarged.

Ovaries in 14-day-old females were not significantly decreased in

weight by testosterone propionate injections, but inhibition was evident histologically for there were fewer large follicles, and the theca and interstitium between the follicles were reduced in amount. Uterine weights were increased 75 per cent but the uterine endometrium was not stimulated to differentiate. Glands were more numerous and conspicuous in control uteri. The clitoris was increased in size.

Autopsy at 18 days of age.

At this age testis weights were not consistently affected by male hormone injection and no histological changes were found. Weights of prostates and seminal vesicles were increased 104 per cent and 494 per cent respectively, and both glands were stimulated histologically. The penis and scrotum of injected males were larger.

In females at 18 days a decrease in ovarian weight of 27 per cent was found (see litter 6). The ovaries, as at 14 days, showed inhibition, with smaller follicles and antra and less interstitial tissue. The uteri were 114 per cent heavier than those of controls but were not stimulated to a functional condition as judged by any effect on the endometrium. No fluid was present in the uterine horns. Glands were found but were not so conspicuous as in control uteri. As at all ages clitorine enlargement was found.

Autopsy at 26 days of age.

The testes of treated males were reduced 16 per cent in weight and showed evidence of damage to the germinal epithelium. Tubule size was considerably reduced and interstitial cells seemed less abundant. A weight increase of 113 per cent occurred in the prostate, but its histological condition was similar to that of the controls except for greater distention of the lumina. The seminal vesicles, however, were precociously stimulated and increased 742 per cent in weight. The penis was slightly larger and the testes had descended into the scrotum in both treated and control males.

In females autopsied at 26 days ovarian weights were not affected but more large follicles were present than in the ovaries of controls. The uteri were 375 per cent heavier, but no functional stimulation was evident, and the most conspicuous change was increase in size. The vagina opened in some of the treated females.

Autopsy at 36 and 56 days of age.

FIG. 1. Testis of a normal 6-day-old male. ×120.

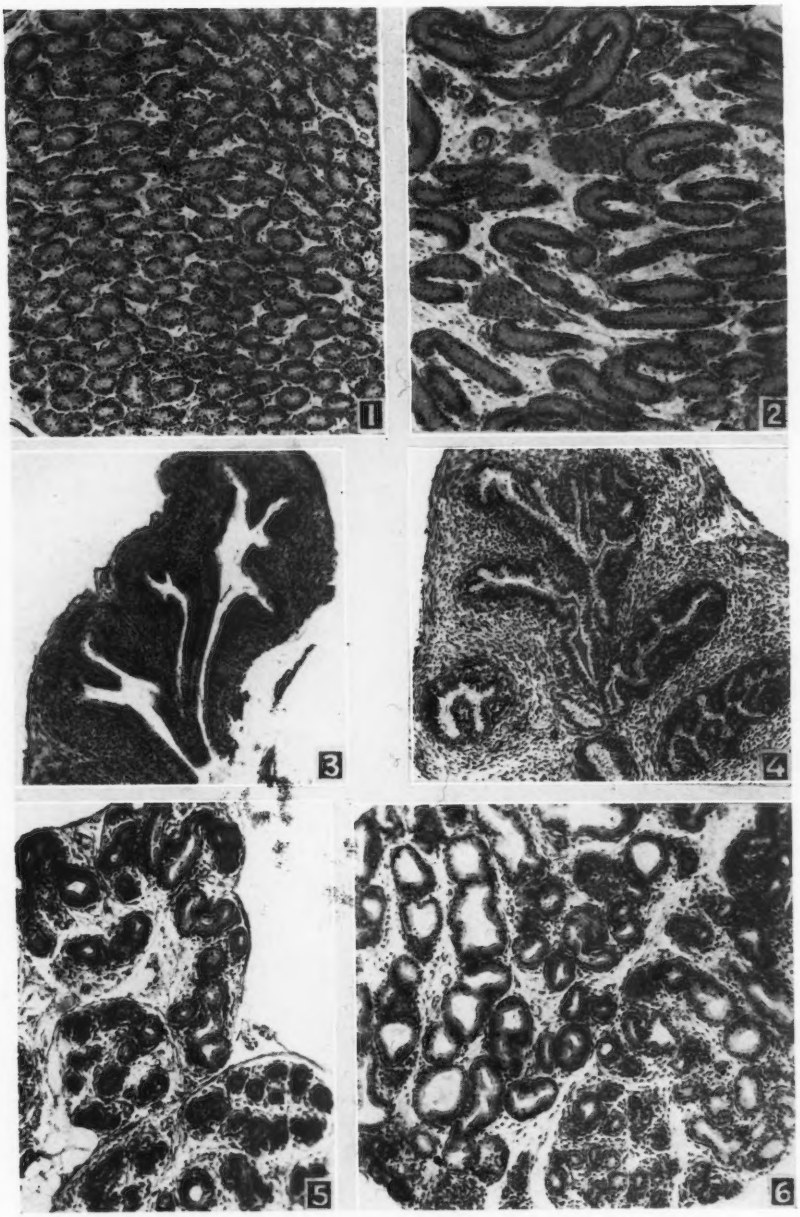
FIG. 2. Testis of a 6-day-old male injected with equine gonadotropin, littermate to control in figure 1. Note groups of interstitial cells. ×120.

FIG. 3. Seminal vesicle of a normal 10-day-old male. ×120.

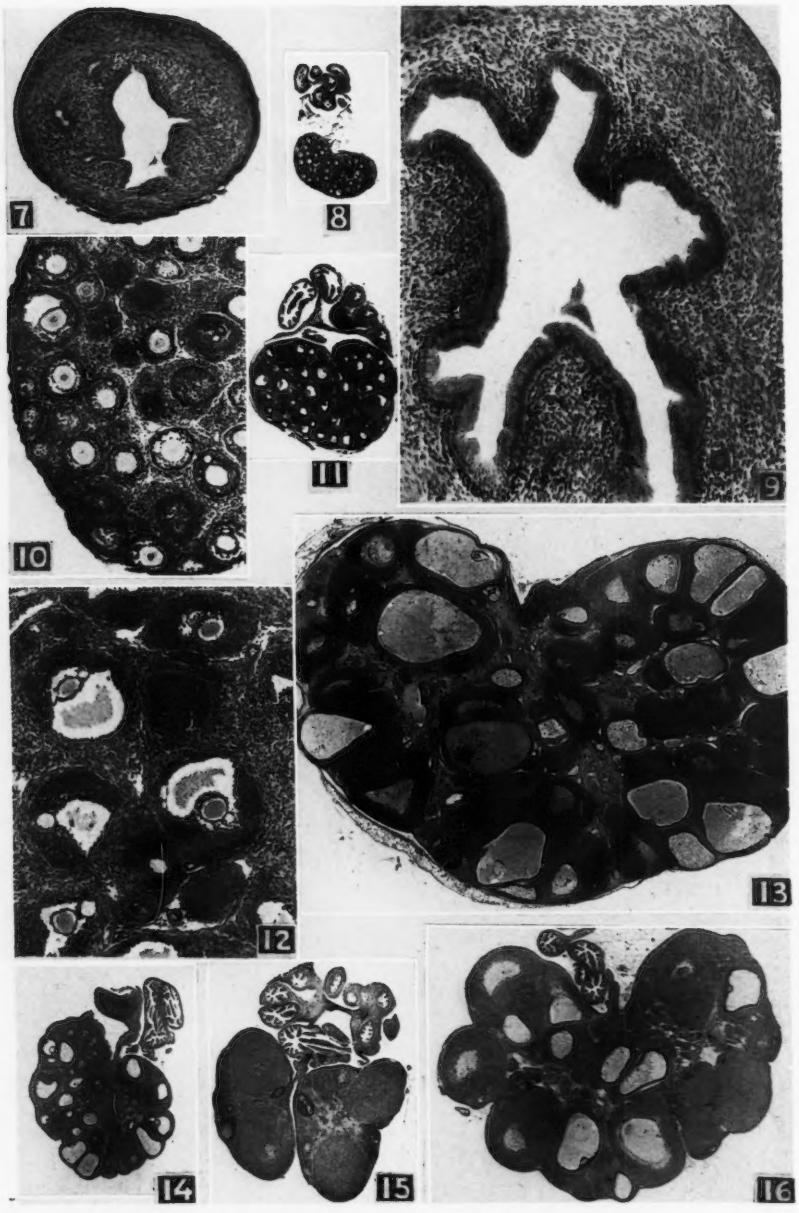
FIG. 4. Seminal vesicle of a 10-day-old male injected with testosterone propionate. Stimulation is evident in the complicated structure and moderately high epithelium. ×120.

FIG. 5. Ventral prostate of a normal 10-day-old male. ×120.

FIG. 6. Ventral prostate of a 10-day-old male injected with testosterone propionate. Acini are more distended than in the control in figure 5; the epithelium is higher and contains cell light areas. ×120.



FIGS. 1–6. See opposite page for description.



FIGS. 7–16. See opposite page for description.

In males killed at 36 days testis weight was decreased 30 per cent and inhibition was apparent histologically. Lumina of tubules were smaller than in control testes, tubular diameter seemed slightly smaller and sperm heads were less abundant. Litter 7 in table 2 gives the organ weights for a litter treated at this age. Prostate weights were 81 per cent heavier than those of controls but the glands were indistinguishable histologically from control prostates. The seminal vesicles were 575 per cent heavier than control glands and were greatly stimulated histologically.

The ovarian weights were unchanged in 36-day-old treated females and no histological changes could be seen. The uteri were 344 per cent heavier but they were not stimulated to functional differentiation. Clitorine enlargement was observed, and the vagina had opened in some treated females.

Testosterone propionate treatment at 56 days caused 13 per cent reduction in testis weights but no histological change was found. Prostates and seminal vesicles were increased 30 per cent and 54 per cent respectively, but histologically they were essentially like the control glands, although the seminal vesicles were in some cases slightly stimulated.

Ovaries of treated females were decreased 13 per cent in weight but there was no change histologically. The uteri were increased 41 per cent in weight but, as at all other ages, size was the only evident histological change. Clitorine enlargement was still found at this age, and the vagina had opened in treated and control females.

These data show that androgenic treatments resulted in gonad inhibition which appeared earlier (between birth and 6 days) in the testis than in the ovary. Increase in the weight of accessory organs was found at 6 days in all except the prostate, and reactivity, as determined by percentage change in weight, increased as age advanced to a maximum at 14 days for the prostate, 18 days for the coagulating

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FIG. 7. Uterus of a normal 18-day-old female. ×120.

FIG. 8. Ovary of the normal 18-day-old female shown in figure 7. ×13.

FIG. 9. Uterus of an 18-day-old female injected with equine gonadotropin, littermate to control in figures 7 and 8. Compare with figure 7 and note increased size, and stimulation of the epithelium. ×120.

FIG. 10. A portion of the normal 18-day-old ovary in figure 8. ×85.

FIG. 11. Ovary of the 18-day-old injected female shown in figure 9. Compare with figure 8. ×13.

FIG. 12. A portion of the ovary of the 18-day-old injected female shown in figure 11. Compare with figure 10 and note stimulation of the theca and follicles. ×85.

FIG. 13. Ovary of a 36-day-old female injected with equine gonadotropin. Compare with figures 11 and 14 and note number and size of corpora lutea and follicles. ×13.

FIG. 14. Ovary of a normal 36-day-old female. ×13.

FIG. 15. Ovary of a normal 56-day-old female. ×13.

FIG. 16. Ovary of a 56-day-old female injected with equine gonadotropin, littermate to control in figure 15. Compare with figure 13 and note number and size of follicles and corpora lutea. ×13.

gland, and 26 days for other organs. Precocious histological differentiation was induced in all accessory reproductive organs except the uterus which responded only by increase in size.

Estradiol benzoate injections.

Autopsy at 6 days of age.

Injection of estradiol benzoate at this age had no significant effect on testis weights but reduced the prostate 13 per cent, and the combined seminal vesicle and coagulating gland weights 22 per cent. The weight changes were small in the accessory glands (see litter 8, table 2) but weight losses were consistent. No histological differences were found between the organs of control and experimental animals.

The weights of combined ovaries and oviducts were increased 55 per cent in 6-day-old females by estrogenic treatment (see litter 8). This was probably because of the response of the oviducts. There was no histological change evident in the ovaries. Uteri were increased 171 per cent in weight and seemed slightly stimulated histologically.

Autopsy at 10 days of age.

All of the weight changes that were either definite or suggested at 6 days in the male were more evident at 10 days autopsy age. Testis weight was reduced 17 per cent, prostate 25 per cent, and seminal vesicles 24 per cent. There was no apparent histological change in the testes. The prostate in injected animals was less well developed histologically and had fewer luminate acini than the glands of controls. The seminal vesicles of the estradiol benzoate-treated males were also inhibited and had less well developed side branches from the central lumen of the glands.

In the treated females the ovaries were 16 per cent lighter in weight but no histological change was found. Uteri were increased 235 per cent in weight. Histological stimulation was evident in the larger lumina, with more mucosal folds, and in the increased height of the epithelium. Glands were beginning to form but were no better developed than in control uteri.

Autopsy at 14 days of age.

At this age testis weights were reduced 16 per cent, prostate 15 per cent and seminal vesicles 20 per cent. No histological changes from the normal condition were found in any of the organs. The penis was smaller in experimental males, indicating a reduction in the amount of male hormone.

A decrease of 18 per cent occurred in ovarian weights of treated females at 14 days. Losses in ovarian weight were found in every litter. The ovaries showed some inhibition histologically in that antra were smaller and were not present in as many follicles as in controls. The theca and interstitium between the follicles were reduced in amount. Uteri were increased 258 per cent in weight, a part of which was fluid content. The endometrium was markedly stimulated. No vaginal opening occurred.

Autopsy at 18 days of age.

Estradiol benzoate injections produced at 18 days a 14 per cent weight reduction in testes, 18 per cent in the prostate and 13 per cent in seminal vesicles (see litter 9, table 2). The only histological changes were found in the testes in which the tubules were slightly reduced in diameter, lumina were smaller and less numerous.

In females the ovaries were decreased 17 per cent in weight and had fewer large follicles, less well developed antra in the follicles and less interstitial tissue than the control ovaries. Uterine weights were 356 per cent greater. Histologically, stimulation was evident but not to the same degree as in 18-day-old females injected with equine gonadotropin. Vaginal opening occurred in some of the treated females.

Autopsy at 26 days of age.

At this age there was a weight reduction in the testis of 27 per cent, prostate 24 per cent, and seminal vesicle 13 per cent. There was indication of damage to testis tubules in that the diameter of the tubules was reduced and the lumina were smaller. The prostate and seminal vesicles were somewhat inhibited histologically. A slight reduction in penis size indicated inhibition of the production of testis hormone.

The ovaries of treated females at 26 days were increased 18 per cent in weight and had larger follicles than the ovaries of controls. The uteri were 440 per cent heavier than in control females. They were distended with fluid and the endometrium showed marked stimulation. No vacuolization of the tall columnar epithelium had occurred. The vagina opened in injected females.

Autopsy at 36 days of age.

At this age the testes of males were reduced only 9 per cent in weight, the prostate 9 per cent and seminal vesicles 22 per cent. Some greater weight reductions were found, as in litter 10, table 2. The only histological change was in the seminal vesicles, in which secretion granules were not so well developed as in controls, indicating some testis inhibition and reduction in male hormone.

The ovaries of treated females were 40 per cent heavier than control ovaries and contained more large follicles; luteinization had begun around degenerating follicles, and in some experimental ovaries corpora lutea were found although none was found at this age in controls. Uteri were increased 365 per cent and, as before, were stimulated histologically. Vaginal opening occurred as the result of treatment and estrous smears were obtained.

Autopsy at 56 days of age.

At this final age, the testes and the accessory glands were not significantly affected in weight and no histological changes were found in any organ.

In injected females the ovaries were not significantly decreased in weight and ovarian histology was unchanged. Uterine weight was in-

creased 31 per cent and advanced differentiation of the endometrium was evident. Vaginal opening had occurred in control and experimental females and cycles were beginning in both groups.

The results of estrogen injections show that the production of testis hormone was inhibited between birth and 6 days, as evidenced

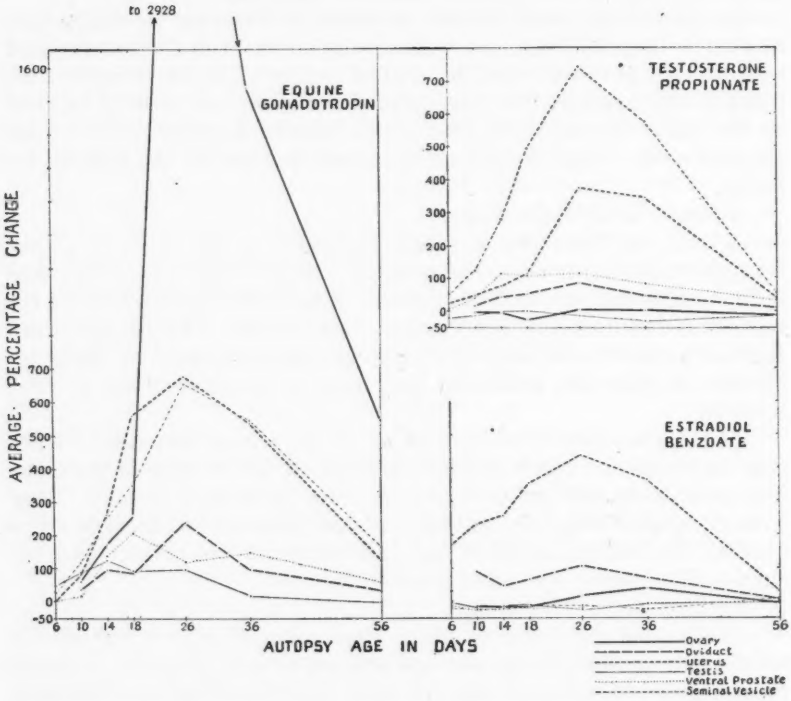

FIG. 17. Curves showing the average percentage change in weight of organs of rats injected for 6 days prior to autopsy, with equine gonadotropin, testosterone propionate and estradiol benzoate.

by reduction in weights of the accessory glands. At succeeding ages greater inhibition in weights and in histology of testes, ovaries and male accessory organs was found. Ovaries were increased in weight and showed histological stimulation at 26 and 36 days of age. The uterus was slightly stimulated at 6 days and more so as age advanced, up to 26 days.

DISCUSSION

The data presented on weights and histological changes in the gonads and accessory reproductive organs in the rat show that the capacity to respond to hormone stimulation developed gradually and

increased up to 26 days of age in the majority of the organs studied. At the age when organs of control animals began to grow most rapidly, the organs of treated animals showed less increase above normal and, in many cases, by 56 days of age there was little or no response. That this is not actually a reduction in reactivity at this age, at least so far as the accessory organs are concerned, has been demonstrated in work to be published elsewhere on the effects produced on the accessory reproductive organs by injection of sex hormone after gonad removal.

The study of weight changes and histological responses induced in the ovary by injection of gonadotropic hormone brings out clearly the lack of reactivity in the ovary of very young rats; the gradual increase in older animals, of weight response associated with thecal hypertrophy and follicular enlargement; and finally, the striking ovarian reactivity at 26 days of age.

The lack of effect of gonadotropic treatment on the very young ovary is in agreement with the results on several species of animals as reported by other workers: Corey (1928), Wiesner (1932), Selye and Collip (1933), Selye, Collip and Thomson (1933) for the rat; Pfeiffer and Hooker (1942) for the mouse; Hertz and Hisaw (1934) for the rabbit; Moore (1941), Moore and Morgan (1943) and Morgan (1943) for the opossum. Our results on thecal hypertrophy in the rat ovary confirm the work of Selye and Collip (1933), Cole (1936), and Marx and Bradbury (1940).

In gonadotropin-injected females killed at 18 days, in our series, both follicles and theca were greatly stimulated but no follicles matured, as reported also by Saunders and Cole (1936). They found, however, that in some rats injected at 19 days ovulation had taken place, and in 21 and 25-day-old rats they obtained ovulation and corpus luteum formation, as reported by Cole (1936). The dosage level used in our work produced the typical picture of great stimulation at 26 days of age, with corpora lutea atretica and large follicles.

Pfeiffer and Hooker (1942) found that in mice (Strong A strain) injected with equine gonadotropin from birth there was no ovarian response at 15 days but by 20 days there was follicular growth, hypertrophy of the theca interna and significant ovarian enlargement. The same histological results were obtained if injections were begun at 21 days of age and the animals were autopsied at 25 and 30 days of age. Injections begun at 35 to 40 days of age produced an increase in follicular growth, supernumerary ovulation and corpora lutea.

The striking change in ovarian weight of the gonadotropin-injected rats at 26 days autopsy age (see fig. 17) and the loss in reactivity at the two subsequent ages of 36 and 56 days present a problem. As reported above, there is an actual decrease in the absolute weight of the ovaries of injected rats from 26 to 36 and 56 days. A change apparently occurs in the ovary at about 20 days, making possible the

maturation and great growth of follicles and the beginning of development of corpora lutea after suitable stimulation. This qualitative change in reactivity is followed by a loss in reactivity that is in large measure quantitative.

A possible explanation of these results may lie in the work of Arai (1920) who measured the diameters of ova in the rat ovary at different ages and counted the total ova, grouping the results into the number of ova less than 20μ in diameter, 20–40μ, 40–60μ, and 60+μ at each age. It was not until the age period of 20 to 26 days that ova of 60+μ were found and at that time there were more (138) such ova than at any other age, the number dropping to 96 at 30 to 36 days, and to 57 at 50 days.

If Arai's measurements can be applied to our results, it seems possible that at least one of the factors normally limiting marked growth and ovulation of follicles may be ovum size. Our treatments did not effect any change in ovum size even though follicles were stimulated to increase in diameter at 18 days. The apparent loss in ability to react after 26 days may be because of the reduction in the number of ova of large diameter. Arai's results show considerable variation; and, although the declining trend is clear, the ovaries of some older animals retain more large ova, which would account for our cases in which very heavy ovaries were induced in older females by gonadotropic treatment.

An analysis of the reactivity of the young ovary to gonadotropic stimulation depends not only upon changes in weight and histology but also upon changes in amount or kind of hormones produced as judged by reactions in the accessory reproductive glands and external genitalia. From 10 days through all ages, the oviducts and uteri registered increased amounts of ovarian hormone by weight increase and histological changes. The fact that both end organs reached a peak of response at 26 days does not indicate that ovarian hormone production was greatest at that age, for injection of both sex hormones, which stimulated the oviducts and uteri directly, produced the same peak indicating greater reactivity in the end organs at that age.

The stimulated ovaries secreted not only additional estrogenic hormone but also androgenic hormone which caused enlargement of the clitoris as reported by Bradbury and Gaensbauer (1939). Our results confirm their findings that clitorine enlargement does not occur, as a result of gonadotropic stimulation, until after 10 days of age.

Injection of sex hormones did not produce such clear-cut changes in responsiveness of the ovary in relation to age as did equine gonadotropin. Testosterone propionate caused only slight reduction in ovarian weights in females killed at 10 and 14 days but at 18 days weights were consistently reduced. No effect was apparent at 26 and 36 days. The reduction that was found at 56 days must be discounted because at that age cycles were beginning, ovarian weights were variable and

responses depended upon the stage of the cycle at which treatment was begun. There is obviously no single trend in change in weight in relation to age. Freeman and Small (1941) also found no significant weight increase with 0.2 mg. of testosterone propionate injected for 3 days in 20 to 25-day-old rats, but with 4 mg. a weight increase of 25 per cent was obtained.

Histological findings help to clarify the results. The first histological change occurred at 14 days when theca and interstitium were reduced in amount and were fibrous in appearance, and by 18 days this was more marked; there were fewer large follicles and antra were smaller. These changes at 18 days were associated with definite weight reduction. At 26 days the ovaries of treated females had a few larger follicles than control ovaries and in two cases a very large cystic follicle was found in experimental ovaries.

These results indicate that inhibition occurred histologically at 14 and 18 days of age and follicular stimulation at 26 days. Permanently damaged ovaries resulting from treatment of young females with androgens have been reported by several workers. Wilson, Young and Hamilton (1940) and Wilson, Hamilton and Young (1941) determined that a total dose of 36 mg. of testosterone propionate given to rats over a 4-week period caused permanent damage to the ovaries only if treatment was begun before the 15th day of life. This would correspond to the early period in which we have shown that testosterone propionate caused ovarian inhibition. Marx and Bradbury (1940) also reported inhibition of theca and interstitium with androgen injections begun on the 8th day of life. It is evident that in the period covering approximately the first two weeks of life the ovary can be inhibited. However, transplantation of such ovaries (produced by injections of testosterone or anterior pituitary-like hormone) into normal females showed that ovulation and corpus luteum formation could take place (Bradbury, 1941). The ovarian effect is secondary, then, to an effect upon the hypophysis as demonstrated by Pfeiffer (1936) by implantation of testes into newborn females.

Stimulating effects of testosterone and testosterone propionate on the older immature rat ovary have been reported when the hormones were administered in large doses over relatively short periods of time. There is ample evidence to show that inhibition of the ovaries of both immature and mature rats is produced after longer periods of injection by inhibition of the gonadotropic hormone of the pituitary (Leonard, Meyer and Hisaw, 1931; Moore and Price, 1932). The stimulation of the ovary after short periods of injection in immature rats is thought by many workers to be produced by stimulation of the production of gonadotropic hormone. Nathanson, Franseen and Sweeney (1938) reported that a single injection of 2.5 to 10 mg. of testosterone propionate resulted in follicle stimulation and corpus luteum formation

in the ovaries of animals weighing 55 to 65 grams. In hypophysectomized rats injections produced no effect on the ovaries. Noble (1939) found follicle stimulation but no luteinization after testosterone propionate administration in 32 to 37 gram rats. Ovaries were reduced in weight. No stimulating effect was evident when the hormone was administered after hypophysectomy.

Pencharz (1940) reported lack of effect of testosterone propionate on the immature rat ovary after hypophysis removal. Hohlweg (1937), Salmon (1938), Clausen and Freudenberger (1939) and Fluhmann (1941) all obtained follicle stimulation, corpus luteum formation or both in immature rats treated with testosterone, testosterone propionate or dehydro-androsterone. Total dosages varied from 2.5 to 45 mg. It is interesting to note that the relatively low dose of 0.1 mg. daily used in our work produced some evidence of follicle stimulation at 26 days. The fact that, in our work, treatment at younger ages caused histological inhibition and reduction in ovarian weight indicates that the hypophysis was inhibited and thus the amount of gonadotropic hormone was reduced. By 20 days some change had taken place so that testosterone propionate stimulated the ovary slightly, presumably by way of the hypophysis. A change in reactivity of the follicles has been demonstrated at that age with equine gonadotropin injections but there is also the possibility of a change in the hypophysis with the assumption of ability to secrete quantitatively or qualitatively different hormone amounts.

Injections of estradiol benzoate also produced inhibition or stimulation of the ovary, depending on the age of the rat at the time of administration. At 10, 14, and 18 days consistent reductions in weight were found. Histological changes appeared at 14 days with reduction in amount of theca and interstitial tissue, and in size of follicles and antra. As with testosterone propionate, injections of estradiol benzoate beginning at 20 days of age did not produce inhibition. In fact, a weight increase of 18 per cent was found. No counts of follicles were made but ovaries of control and injected females appeared similar histologically. However, at 36 days of age when the weight increase averaged 40 per cent, corpora lutea were found in the ovaries of some of the injected animals, showing the stimulating effect of the estrogen. No corpora lutea appeared in control ovaries at this age.

Inhibition of very young ovaries with estrogen treatments has been reported by other workers. Marx and Bradbury (1940) obtained inhibition of theca and interstitium with injections begun at 8 days. Weichert and Kerrigan (1942) injected lactating females with estrogens beginning at parturition and found great inhibition of the ovaries of suckling females with respect to follicle size and antrum formation at 14 days of age. No weights were taken. Several workers have reported permanent damaging effects on ovaries with estrogen treatment soon after birth. Turner (1941) produced abnormal ovaries in adults by injecting estrogen the first 10 days of life. Wilson (1943)

found that if injections were begun on the 1st, 5th, or 10th day after birth (treatments given 3 times a week for 4 weeks) ovaries were small and, while some were normal histologically, many were atretic. If injections were begun later than 10 days (15, 20, 30 or 40) ovaries were normal at autopsy.

Wilson's analysis agrees with our findings that estrogen treatment caused inhibition in the very young ovary, but that at 20 days or later no inhibition was produced.

Lane (1935b) injected estrin into 22-day-old rats and found that after 5 days of treatment the ovaries increased 26.8 per cent in total follicle count and contained 50 per cent more vesicular follicles. With increased periods of injection the follicle count fell below normal but the percentage of vesicular follicles remained high. Lane concluded that estrin stimulated the hypophyses to secrete additional gonadotropic hormones and later there was an inhibition of liberation of follicle stimulating hormone, and stimulation of luteinizing hormone or something associated with it. This is based upon the idea that follicle stimulating hormone increases the total number of follicles while luteinizing hormone causes development of antra (Lane, 1935a). This was demonstrated in hypophysectomized rats (Lane and Greep, 1935). From this work it seems possible that the increase in ovarian weight in our experiments at 26 days may have resulted from an increase in the number of follicles.

The production of corpora lutea in immature rats by estrogens has been reported by Hohlweg (1934, 1936) and Hohlweg and Chamorro (1937). It was found that doses of as low as 4 μg. of estradiol benzoate resulted in the production of corpora lutea in rats approximately 6 weeks of age (50 grams in body weight). Larger doses were also used but were not found necessary. If the hypophysis was removed two days after the injection of estrogen, no corpora lutea were found; but if extirpation did not take place until 4 days after the administration of hormone, corpora lutea resulted (Hohlweg and Chamorro, 1937). They concluded that injections of follicular hormone stimulated (directly or indirectly) production of luteinizing hormone from the hypophysis. Merckel and Nelson (1940) were unable to cause corpus luteum formation in immature rats with single or repeated massive doses of estradiol benzoate. The formation and maintenance of corpora lutea in adult female rats by estrogen injections have been reported by a number of workers (Nelson, 1935; Wolfe, 1935; Selye, Collip and Thomson, 1935). It may be concluded that estrogens in our work inhibited the young ovary before 20 days, indirectly, by inhibition of the hypophysis. After that age stimulation of the ovary took place, probably by means of the hypophysis, although direct stimulating effects of estrogens upon the ovary of hypophysectomized rats have been reported by Pencharz (1940), Williams (1940) and Simpson, Evans, Fraenkel-Conrat and Li (1941).

Unfortunately no conclusions as to the production of hormone in

ovaries of treated females can be drawn from uterine reactions because both estradiol benzoate and testosterone propionate stimulated the uterus directly.

With all substances administered the uterus showed a definite reactivity in weight related to age, with a peak at 26 days. Stimulation of the uterine mucosa was found at 10 days with injections of equine gonadotropin and estradiol benzoate and at subsequent ages precocious differentiation of the endometrium occurred and the uteri were distended with fluid. Testosterone propionate injections produced mainly an increase in size at all ages, and no functional development of the endometrium was induced. The dosages of testosterone propionate and estradiol benzoate which we employed did not cause abnormal uterine differentiation as reported by several workers using larger doses for longer periods of time.

Freeman and Small (1941) studied changes in organ weights and reported that the uterus of young rats was the most sensitive "target" organ for testosterone propionate. In our experiments, the uterus was less reactive than the seminal vesicle. Freeman and Small weighed the seminal vesicles "with all lobes of the prostate" and thus found the combination less sensitive since the prostate does not have such a high relative reactivity and undoubtedly masked the seminal vesicle response.

The male organs followed the reaction pattern of those of the female in most respects. The testis responded to gonadotropic treatment earlier than the ovaries and was increased in weight at 6 days and showed the development of clumps of interstitial cells. Interstitial cell hypertrophy has been reported for older prepuberal animals after treatments with pituitary implants, and substances from the urine of pregnant women and the blood serum of pregnant mares (Moore and Price, 1931; Cole, Guilbert and Goss, 1932; Price, 1936; Cole, 1936 and others). A peak in percentage increase in weight was reached early, at 14 days, and declined slowly. No stimulation of spermatogenesis was found, in agreement with the work of many others, but tubule size and lumen size were increased at 18 days.

The production of hormone was increased in stimulated testes at all ages, as reflected in increase in weights of the accessory glands. Since the reactivity of the prostate and seminal vesicle increased with age, it was impossible to make any estimate of the relative amounts of hormone produced at different ages by stimulation of the testes.

Testosterone propionate injections did not produce a graded reduction in testis weights associated with age. Definite and clear-cut reductions were obtained at all ages except 14 and 18 days. Moore and Price (1938) showed that testosterone propionate injections caused injury to the testes of young males in inverse proportion to the dosage. In the present work, it was thought that at 14 and 18 days, the daily dose of 0.1 mg. might be high and therefore not give inhibi-

tion. To test this, daily doses of 0.025 mg. and 0.010 mg. were given and both produced a reduction of approximately 24 per cent showing that the lack of effect of 0.1 mg. was related in some way to a change in reactivity of the testis or hypophysis. This point requires further analysis at these ages. Damage to the germinal epithelium and reduction in lumen size were obtained at 18 days when weights were not significantly affected. Weight reduction and histological inhibition were attributed to inhibition of the gonadotropic function of the pituitary.

Injection of estradiol benzoate caused reduction of testis weight at all ages but it was too slight to be considered significant at 6, 36 and 56 days. At the other four ages, consistent weight decreases were found. Some damage to testis tubules was found but spermatogenesis was not affected. Weichert and Kerrigan (1942) reported smaller testes and smaller tubular diameters in 14-day-old males whose mothers were injected with estrogens beginning at parturition. Accessory reproductive glands were inhibited in histological development.

Hormone production of the testes in our estrogen-injected animals was definitely reduced at all ages except 56 days as shown by reduced weights of the accessory glands. No direct stimulating effect was found on the muscle and connective tissue of accessory organs with the dose used. Some histological inhibition was found in the seminal vesicle but only slight evidence in the prostate, which has been found capable of developing to a certain degree without the presence of testis hormone (Price, 1936).

All of the male accessory glands studied responded to stimulation with increased endogenous, or exogenous male hormone in direct relation to increasing age, up to a peak which was reached at 18 days for the prostate and 26 for the seminal vesicle and coagulating gland. The last two (together) responded at 6 days. At 10 days both prostate and seminal vesicle were stimulated to precocious histological development. Hooker (1942) has stressed the relation of age to reactivity in the accessory reproductive organs of castrated male rats. He found a great increase in sensitivity to androgen treatment in seminal vesicle epithelium at about 40 days of age. This was maintained at 50 and 60 days but at 80 days reactivity had returned to the prepuberal level.

A relationship between age and the development of estrous responses has been demonstrated by Wilson and Young (1941) who studied mating behavior in guinea pigs and rats spayed at birth. They administered estrous-inducing hormones at various ages and found that mating responses could not be induced in the guinea pig until 15 days of age and that a threshold comparable to that of the adult was not reached until 30 days of age. In rats tested at 20 and 30 days and 10 months a few responded at 20 days but at 30 days a high sensitivity was reached which was maintained at 10 months.

SUMMARY

Equine gonadotropin, testosterone propionate, and estradiol benzoate were injected into female and male rats for periods of 6 days beginning at birth, 4, 8, 12, 20, 30 or 50 days. Studies were made of the changes in weight and histology of the gonads and accessory reproductive organs.

Reactivity, as judged by increase or decrease in organ weights in response to hormone treatment, was present between birth and 6 days of age in the testis, prostate, seminal vesicle, coagulating gland and uterus. Ovarian weight response occurred in the period between 4 and 10 days of age.

The initial percentage change in weight in response to stimulation was small in every organ but increased with age to a peak which was reached at 14 days for the testis, between 14 and 18 days for the prostate, and at 26 days of age for all other organs studied.

Capacity to respond to stimulation histologically by precocious differentiation also developed gradually, and was found at younger ages than the greatest weight increase.

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THE EFFECT OF ESTROGENS ON THE PERIPHERAL BLOOD AND BONE MARROW OF MICE

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THE OBSERVATION by Kyes and Potter (1934) that female pigeons had a cyclic ossification of the marrow spaces associated with the maturation of the ovarian follicle has been the basis for much research work upon the effect of estrogens and androgens on calcification and ossification in various species of organisms. Zondek (1937) first observed that when mice were given injections of estrogens in large amounts the marrow spaces were invaded and largely replaced by bone. Arnold (1937) and later Tyslowitz (1941), Castrodale and his coworkers (1941) and Crafts (1941) demonstrated that large doses of estrogens caused a severe anemia, purpura and death in both male and female dogs. No change of significance was noted by Tyslowitz (1941) in the peripheral blood picture of the rhesus monkey after prolonged administration of large doses of estrogens. Crafts (1941) noted only a slight anemia in the rhesus monkey after the administration of 10 mg. of stilbesterol daily over an extended period. On the other hand he found that giving only 5 mg. daily to dogs produced rapid and marked effect on the hemopoietic system. Vollmer and Gordon (1941) observed a lowering of the erythrocyte count in castrated female rats after administration of estradiol benzoate. No careful study of the effects of estrogens on the cellular elements of the peripheral blood have been made in the species of organism in whom the most spectacular proliferation of endostial bone and subsequent crowding of the bone marrow spaces occurs, namely, mice and fowl. Domm (1943) and his associates, studying the brown leghorn fowl, concluded that the lower erythrocyte count occurring in the female brown leghorn fowl might be due to the increase in endostial bone and the resulting mechanical interference with erythropoiesis.

Gardner and Pfeiffer (1938) and Wentworth, Smith and Gardner (1940) have studied in detail the gross and microscopic effects of estrogens on the bones of mice. The following study was undertaken in an attempt to determine whether this marked endostial proliferation resulting from estrogen injection in mice had any appreciable effect on the cellular elements of the peripheral blood.

Received for publication January 14, 1944.

MATERIAL AND METHODS

Sixty-five white mice, all weighing approximately 20 grams (age six to eight weeks), were placed on a regular laboratory diet found to be satisfactory in our animal quarters in the past. After two weeks, control peripheral blood determinations were made on each mouse. Blood sampling was made by snipping off the end of the tail and using, insofar as was possible, a free flow of blood. When sampling was complete the bleeding was controlled by gentle finger pressure and application of collodion or Monsel's solution.[1] The determinations on the peripheral blood usually included: total leucocytes, erythrocyte count, platelets, hemoglobin and a differential count made on dry smears prepared with Wright's stain.

Estradiol benzoate[2] in sesame oil was injected intramuscularly in a group of forty male and female mice controlled as above. Injections of estradiol benzoate in sesame oil were given weekly in amounts not less than 16 micrograms for a period of thirty-six weeks. Two control groups were given equivalent volumes of sesame oil alone or normal saline respectively (table 1).

The determinations on the peripheral blood were repeated on each animal at intervals of six weeks until the termination of the experiment except in one group (table 1) in whom control blood studies were made at the beginning of the experiment and at the close of the experimental period only. This latter procedure was followed to determine what effect, if any, the bleeding for sampling at intervals of six weeks would have upon the blood count.

At the end of thirty-six weeks all animals were sacrificed, representative tissues fixed in Zenker formol solution, paraffin imbedded and stained with hematoxylin eosin and azure. The organs and tissues studied microscopically were: bone and bone marrow, liver, kidneys, lymph-nodes, spleen, adrenals and lungs. Long bones were examined grossly at the time the animal was sacrificed and sectioned longitudinally and transversely after decalcification.

RESULTS

Effects of estrogens on the cellular elements of the peripheral blood. No appreciable effect was noted on the platelet count during or at the conclusion of the experimental period. Except for individual variation no change of any magnitude was noted in the average erythrocyte count (table 1). It will be noted, however, that the average erythrocyte count of both the estrogen injected and control group was lower at the close of the experiment than the average pre-injection count, and that the decrease in the former was slightly greater.

The average leucocyte count was higher at the termination of the experiment in both the estrogen injected and control animals (table 1). This elevation was greater in the estrogen treated group.

Except for a slight increase in the average per cent and absolute number of lymphocytes in the estrogen treated group as opposed to the control group no effect of significance was noted on the differential blood picture (table 1).

[1] Ferric subsulphate.

[2] Estradiol benzoate (Progynon B) was obtained through the courtesy of Dr. Erwin Schwenk of the Research Division of the Schering Corporation, Bloomfield, New Jersey.

TABLE 1. AVERAGE BLOOD COUNTS OF ESTROGEN TREATED AND CONTROL MALE AND FEMALE MICE

Number of Mice	Duration of Experiment	Treatment: Intramuscular or Intraperitoneal	Frequency of Blood Counts	Average Blood Count Male and Female*									
				W.B.C.**	Hgbln	R.B.C.	Plat.	Polys	Lymphs	Mono	EOS	BAS	
5	36 weeks	sesame oil only weekly	At beginning of experiment and at close of experiment only	8.1	14.4	9.8	10.1	23	69	5	3	>1	control count before beginning of experiment
				8.8	13.8	9.5	9.6	20	70	7	2	1	count at close of experiment
10	36 weeks	sesame oil only weekly	Every six weeks	8.5	14.6	10.0	10.5	23.5	65	8	3	>1	control count
				9.8	14.1	9.3	9.9	21.0	67	8.5	3	0.5	count at close of experiment
10	36 weeks	sterile saline only weekly	Every six weeks	9.2	14.2	10.4	9.4	28.0	63	6	2	1	control count
				11.0	15.1	9.4	9.8	24.0	66	8	1.5	0.5	count at close of experiment
40	36 weeks	estradiol benzoate in sesame oil 16+ micromicrograms weekly	Every six weeks	7.9	14.2	9.7	9.5	26.0	65	7	2	>1	control count
				12.3	14.1	8.6	10.3	17.0	72	8	2	>1	count at close of experiment

* No appreciable difference noted in blood counts on male and female mice either before or at the close of experiment.

** Abbreviations:

W.B.C. Leucocytes per mm³ in thousands.
Hgbln Hemoglobin in grams per 100 cc. photoelectric Hemoglobinometer.
R.B.C. Erythrocytes per mm³ expressed in millions.
Plat. Platelets per mm³ expressed in hundred thousands.
Polys Per cent neutrophiles.
Lymphs Per cent lymphocytes.
Mono Per cent monocytes.
EOS Per cent eosinophils.
BAS Per cent basophils.

No significant effect on the hemoglobin content of the peripheral blood occurred in either the estrogen injected or control animals.

Effect on gross and fixed tissue studied. Bone and bone marrow: Grossly the bones of animals receiving estrogens over this extended period showed a marked increase in calcification. This was recognizable on longitudinal section to the naked eye. The bone marrow spaces appeared in many specimens to be almost entirely replaced by compact bone. Histologically, however, a considerable amount of active bone marrow remained between the spicules of bone produced by endostial proliferation. The hemopoietic tissue in all instances appeared entirely normal or erythropoiesis more intense than in the normal control groups in whom no bony proliferation encroached on the marrow spaces.

The spleen and liver. The spleens of the estrogen and non-estrogen injected animals were not weighed. However, there appeared to be no gross difference in size in the spleens of the estrogen and normal controls. Histologically, however, erythropoiesis appeared more intense in the estrogen treated group. This was true also of the liver.

All other organs and tissues examined histologically, namely, adrenals, lymph-nodes and lungs appeared normal as compared to the controls.

No essential difference was noted in the degree of endostial bony proliferation in the male and female mice treated with estrogens. There was likewise no difference in the effect on the peripheral blood count.

In the control group of mice who received sesame oil or sterile saline injections no abnormalities in the blood count or histological appearance of the bone and other tissues or organs were noted that were considered attributable to the treatment.

COMMENT

In spite of the partial replacement of the bone marrow spaces by endostial new bone formation in mice (male or female) treated with large doses of estrogens no marked alteration in the peripheral blood count has resulted. The moderate reduction in the average erythrocyte count for the group of estrogen treated animals may be significant. The fact that anemia was not prominent is probably due to the fact that normal or hyperplastic marrow exists between the spicules of invading new bone and in addition extra medullary hemopoiesis is more marked than normal in the spleen and liver.

SUMMARY

In male and female mice treated with estradiol benzoate sufficient to produce a partial replacement of the bone marrow cavities by endostial new bone formation an anemia of only questionable significance was noted.

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THE UPTAKE OF RADIOACTIVE IODINE BY THE THYROIDS OF RATS MADE GOITEROUS BY POTASSIUM THIOCYANATE AND BY THIOURACIL[1]

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RECENT reports by the MacKenzies (1943) and by Astwood *et al.* (1943 a and b) on the goitrogenic action of certain sulfonamides and thiourea-like drugs have provided a new approach to the study of thyroid physiology. These agents were found not only to produce goiters but also to lower the metabolic rates of experimental animals. The apparent thyroid depressing action of these agents led Astwood (1943c) to use thiourea and thiouracil in treating thyrotoxic patients. He observed a definite lowering of the metabolic rates of such thyrotoxic patients. An understanding of the mode of action of these thyroid depressants would be of real and practical value to the clinician who treats abnormal thyroid states. Such an understanding may also lead to important fundamental information concerning the physiology of the normal thyroid gland and may reveal something of the pathologic physiology of Graves' disease.

For more than a decade it has been known that certain nitriles have a definite depressing effect on the metabolic rates of some experimental animals and that such depressed metabolic rates are associated with the development of thyroid hyperplasia. This goitrogenic effect of the nitriles is prevented by the coadministration of iodine.

Astwood (1943) has investigated a large number of chemical compounds for their goitrogenic effect. He has found in general two chemical groups which depress thyroid function and cause thyroid hyperplasia. More than twenty compounds having a structural grouping which contained NH·CS·NH were goiter producing. He has also found a dozen drugs, including several sulfonamides which have an aniline group as a structure common to all, to be goitrogenic. He failed

Received for publication January 17, 1944.

[1] Aided in part by grants from the Josiah Macy, Jr. Foundation, the H. N. C. gift, Harvard University, and the John and Mary R. Markle Foundation. The thiouracil used was kindly furnished by Lederle Laboratories, Inc.

[2] Henry P. Walcott Fellow, Harvard University.

to produce any thyroid enlargement in rats treated with organic cyanides. Potassium thiocyanate did produce goiters in rats maintained on the drug for some time. These goiters could be prevented by added iodine. The goiters produced by the derivatives of thiourea and aniline could not be prevented by iodine. They could be prevented only by the administration of thyroxine or of desiccated thyroid. Diiodotyrosine had no preventive effect. These observations led Astwood (1943a) to postulate that these new groups of thyroid inhibitors act through a competitive mechanism in the enzyme system responsible for the conversion of diiodotyrosine to thyroxine.

Of fundamental interest is the observation that the derivatives of thiourea and aniline produce goiters despite the administration of iodine in excess of the amount needed to prevent the action of other known goitrogens. In an attempt to explain the different effects of iodine upon these various goitrogens, and to throw further light upon the action of some of these agents the present study of the uptake of radioactive iodine by thyroids made goitrous by iodine inhibited and iodine resistant goitrogens was undertaken.

Potassium thiocyanate was used as an iodine inhibited goitrogen and thiouracil was used as an iodine resistant goitrogen. The uptake of radioactive iodine, a technic of study devised by Hertz, Roberts and Evans (1938) with minor modifications devised by Peacock (1943), was used as a method of determining the ability of such treated thyroids to absorb iodine.

PROCEDURE

Seventy five male rats of the Sherman strain weighing between 130 and 170 grams were placed on a basal diet with a supplement of iodine in the drinking water. The basal diet was the rachitogenic diet #2965 of Steenboch and Black (1925) and consisted of yellow corn, 76%, wheat gluten, 20%, calcium carbonate, 3%, sodium chloride, 1%. To prevent the development of rickets there was added to each kilogram of the above diet mixture four grams of irradiated yeast powder. In the absence of supplemented iodine this diet was found by Kraus and Monroe (1930) to be goitrogenic. The diet mixture was found to contain approximately 15 gamma of iodine per kilogram of diet. Iodine was supplemented by incorporating it as potassium iodide in the triple distilled drinking water so as to give each animal taking a normal amount of drinking water about .60 micrograms of iodine daily. This daily intake of iodine has been demonstrated (Levine, Remington and von Kolnitz, 1933a & b) to cause some minimal enlargement of the thyroids of animals maintained on such a diet. Such a borderline goitrogenic diet was chosen so that the potassium thiocyanate treated animals would not have the benefit of iodine prophylaxis. The animals were maintained for two weeks on this basal diet before any other positive agents were added to the intake in order to permit acclimatization to the rather severe dietary change. The animals were weighed two times each week. Two weeks after the animals had been first placed on the basal diet 25 animals were given 0.1% thiouracil in the drinking water. Another 25 animals were given 0.25% of

potassium thiocyanate in the drinking water. The remaining 25 animals were maintained on the basal diet alone. On the 28th day after starting the thiouracil and thiocyanate treatment, all of the animals were injected intraperitoneally with one cc. of a solution containing 2.5 micrograms of radioactive iodine I^{131} as sodium iodide. Four hours later all of the animals were killed by means of ether. The thyroid glands were removed and weighed. One lobe of each thyroid was fixed in 10% neutral formalin and then prepared for histologic study. The remaining lobe of each thyroid was weighed again and placed in 3 cc. of 2% NaOH in order to digest the thyroid tissue for convenience in analysis. The radioactivity of aliquots of these solutions were compared with that of an equal aliquot of the original medium injected into the animals in order to determine the percentage uptake of the labelled iodine.

RESULTS

The gain in weight was poor in all animals maintained on the diet used in this study. The difference in average gain of weight of the

TABLE 1. COMPARISON OF INITIAL & FINAL BODY WEIGHTS, MEAN WEIGHTS OF THYROID GLANDS AT AUTOPSY, AND OF MEAN PERCENTAGE OF ADMINISTERED RADIO IODINE IN THYROID GLANDS

Groups of Animals	Average Initial Body Weight in Grams	Average Final Body Weight in Grams	Mean Thyroid Weights in Mgm.	S.E.$_M$	Mean % Uptake of Administered Radioactive Iodine	S.E.$_M$
Control	146.4	160.5	15.9	∓1.22	55.7	∓3.94
Thiocyanate treated	143.7	159.4	29.3	∓1.54	86.5	∓2.83
Thiouracil treated	144.0	154.0	49.5	∓2.66	10.1	∓0.27

three groups of animals was less than 4% of the average body weight at the beginning of the experiment. The animals treated with either the thiouracil or with the potassium thiocyanate demonstrated no change in activity nor in quantity of food consumed.

At autopsy no gross organ changes were observed other than those to be described in the thyroid glands. X-ray studies of representative animals failed to demonstrate any bone changes characteristic of rickets. No exophthalmos was observed during life nor at the time of autopsy.

The thyroid glands of the control animals were normally pale and to the naked eye were not enlarged. The weights of these glands were somewhat greater than those usually observed in normal rats (10 mgm) (see table 1). The weights of these thyroid glands varied between 9.2 and 42 mgms. and averaged 15.9 mgms. Animals treated with potassium thiocyanate had thyroid glands which were dark red, obviously quite vascular, and were grossly enlarged. The weights of the thyroid glands of these animals varied between 17.6 and 41.2 mgms., and averaged 29.3 mgms. The thyroid glands of the animals

treated with thiouracil were very large, dark red and vascular. The weights of this group of thyroids averaged 49.5 mgms. and varied between 25.0 and 85.0 mgms.

The histologic picture of the thyroid glands of control rats varied from minimal hyperplasia to no hyperplasia at all. This is what one would expect to observe in animals maintained on a borderline iodine deficient intake. The acinar cells were either normal or slightly increased over the normal in height. There were no papillary projections

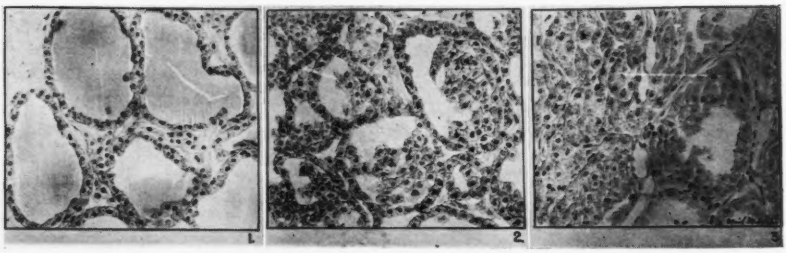

FIG. 1. Camera lucida drawings of rat thyroids. (1) Control, (2) Thiocyanate treated, (3) Thiouracil treated.

of epithelial tissue into the follicles. Except for peripheral vacuolization of the colloid in some instances the lumina of most follicles contained a normal amount of colloid. A marked hyperplasia was observed in the thyroid sections of animals which had been treated with potassium thiocyanate. There was a complete loss of colloid in all sections. The acinar cell height was greatly increased over the normal, and in all sections the papillary projections were marked. The hyperplasia observed in the thyroids of animals treated with thiouracil was even greater. The acinar cells had even a greater height than did the thiocyanate treated thyroids. The colloid was completely lost in all sections. Indeed in many instances the lumina of the acini were completely occupied with rows of epithelial tissue so as to suggest the strands of epithelial cells observed in fetal thyroids.

The differences in the uptake of labeled iodine were very definite with practically no overlapping from one group to another. The control rat thyroids were found to contain between 20 and 85 per cent of the radioactivity present in the originally administered labeled iodine. The average uptake of the radioactive iodine in this group of thyroids was 56%.

Thyroids of the potassium thiocyanate treated rats took up an average of 86% of the administered labeled iodine. The range of observed radioactivity in these thyroids made goitrous by the ingestion

of potassium thiocyanate was between 64 and 110%* of the activity contained in the originally injected samples. The thiouracil treated rat thyroids were found to contain between 5 and 22% of the administered labeled iodine. The average radioactivity observed in this group of thyroids was 10% of the original sample.

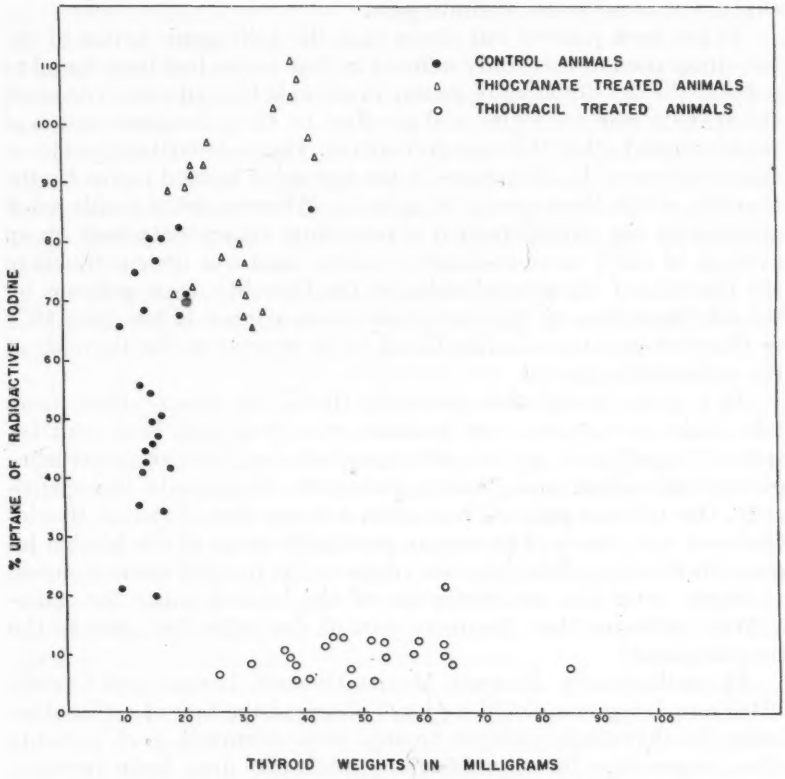

FIG. 2. Collection of radioactive iodine by rat thyroids, referred to weight.

DISCUSSION

It is evident from the above results that potassium thiocyanate and thiouracil have a goitrogenic effect. The average weight of the thyroids of potassium thiocyanate treated rats was 185% greater than that of the control rats; whereas the average thyroid weight of the thiouracil treated rats was 310% greater than that of the untreated

* The average probable error of this technic is about 8% so that individual values over 100% merely represent the expected fluctuations in the measurements.

animals. The hyperplasia as evidenced by the histologic examination was even greater in the thyroids of the thiouracil treated animals than in the thyroids of the thiocyanate treated animals. The thyroid hyperplasia of both groups of animals was severe enough so that had it been associated with hyperfunction of the thyroid, there would have been a definite and rather severe weight loss. Instead there was a slight, but nonetheless, definite gain.

It has been pointed out above that the goitrogenic action of the two drugs used in this study differed in that iodine had been found to prevent the development of goiters in animals treated with potassium thiocyanate whereas iodine had no effect on the goitrogenic action of thiouracil and other thiourea derivatives. The most striking results in this study were the differences in the uptake of labeled iodine by the thyroids of the three groups of animals. Whereas the thyroids made goitrous by the administration of potassium thiocyanate took up an average of 153% more radioactive iodine than was demonstrated in the thyroids of the control animals, the thyroids made goitrous by the administration of thiouracil took up an average of less than 18% of the average labeled iodine found to be present in the thyroids of the untreated animals.

In a recent publication (Rawson, Hertz and Means, 1943) from this clinic we reported two patients who developed large and extremely hyperplastic goiters with symptoms and laboratory evidence of hypothyroidism while taking potassium thiocyanate therapeutically. One of these patients was given a tracer dose of radioactive iodine and was observed to excrete practically none of the labeled iodine. Furthermore determinations done on the thyroid tissue removed at biopsy after the administration of the labeled iodine for radioactivity indicated that the major part of the iodine had gone to the thyroid gland.

In another study (Rawson, Means, Peacock, Lerman and Cortell, 1944) now being completed we have observed the fate of radioactive iodine in thyrotoxic patients treated with thiouracil. Such patients after preparation for thyroidectomy with this drug have received tracer doses of labeled iodine. A large percentage of the administered iodine has been observed to be excreted in the urine during the first 24 hour period following its administration. Thyroid tissue removed 24 hours after administration of the tracer iodine has been found to contain relatively little radioactivity. The differences observed in the uptake of labeled iodine by the three groups of experimental animals as well as clinical observations made on patients treated with thiouracil or made goitrous by the administration of potassium thiocyanate would appear to be fundamental, and may direct our attention to an eventual understanding of the modus operandi of both agents.

Good evidence has been produced that the goiters in animals treated with derivatives of thiourea or aniline develop as a direct

result of an increased tropic action of the thyroid stimulating hormone (TSH). For example, both the MacKenzies (1943) and Astwood *et al.* (1943b) have demonstrated that goiters will not develop in animals treated with these drugs following hypophysectomy. The MacKenzies have also reported that the pituitaries of rats made goitrous by these drugs present the same histologic picture as do the pituitaries of thyroidectomized rats. It is thought that the goiters of potassium thiocyanate treated animals are also the direct result of pituitary thyrotropic stimulation though there is no experimental evidence that such goiters will not develop in the hypophysectomized animals.

The increased activity or secretion of the hypophyseal thyroid stimulating hormone (TSH) is thought to be brought about either by a decreased basal oxygen consumption of the peripheral cells of the body or as a result of a decreased output of normal thyroid hormone.

In the recent paper (Rawson, Hertz and Means, 1943) from this clinic concerning the occurrence of goiters in patients being treated for hypertension with potassium thiocyanate we cited evidence in favor of the theory that the symptoms and findings of hypothyroidism were due to some interference with the manufacture of the thyroid hormone, *i.e.*, the blood iodines were of the level observed in patients with true myxedema, and that the administration of thyroid to patients receiving potassium thiocyanate cause a disappearance of the symptoms of myxedema and a rise in the basal metabolic rate with disappearance of the goiters. Dempsey and Astwood have cited evidence that the drugs used by them acted to interfere with the elaboration of the thyroid hormone. They treated animals receiving these agents with thyroxine and were able not only to prevent the development of goiters but also to cause goiters which had already occurred as the result of treatment with these chemical agents, to disappear, even though the administration of the goitrogen was continued.

It is thought by several investigators (Marine, 1935, Means, 1939, Uotila, 1940) that there exists a rather fine balance between the pituitary and the thyroid, designated by Salter (1940) the "pituitary-thyroid axis." The balance between the members of this axis is thought to depend on the blood levels of thyroid hormone and thyroid stimulating hormone. Any deficiency of the thyroid hormone is believed to cause an increase in the amount of thyroid stimulating hormone liberated by the pituitary. If this fine balance actually exists, one could well expect the diminished titer of thyroid hormone which results from the administration of the goitrogenic agents to result in a prompt liberation of an increased quantity of TSH and the subsequent development of goiters. See Figure 3.

To understand then the specific action of these drugs in producing goiters one should understand the various steps in the natural forma-

tion of the thyroid hormone and just how these drugs interfere with such steps in the natural syntheses. Our knowledge of the cellular physiology of the thyroid is certainly too limited to permit us to state where either of these drugs impinges on the formation of thyroid hormone. Of the potassium thiocyanate we are able at present only to say that the manufacture of thyroid hormone is diminished and that goiters resulting from treatment with this agent have a greater than normal avidity for iodine.

Of thiouracil we are able to say that it acts to block the usual

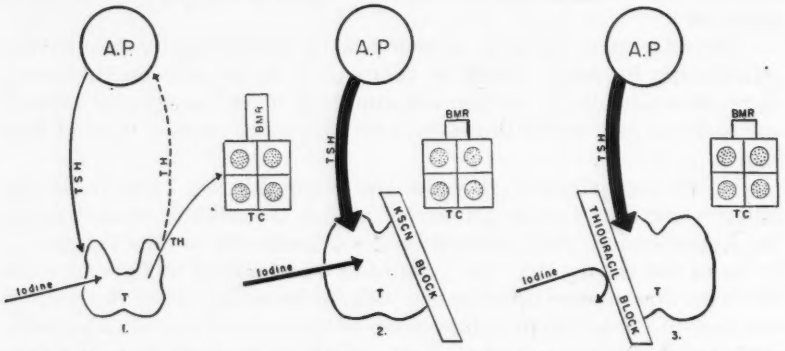

FIG. 3. Diagrammatic representation of the action of thiocyanate and thiouracil upon the thyroid.

1. Normal situation. The anterior pituitary—AP—by means of its hormone—TSH—and in the presence of an adequate supply of iodine stimulates the thyroid—T—to produce its hormone—TH—which stimulates the metabolism of tissue cells—TC—and inhibits AP.
2. Thiocyanate imposes an obstruction to the completion of TH at some point in the chain of hormone synthesis distal to the uptake of iodine. No active TH is put out and consequently the BMR falls. The uninhibited AP produces an excess of TSH. This causes hyperplasia of T and increased uptake of iodine.
3. Thiouracil causes an obstruction to the uptake of iodine. Again no active TH is put out and BMR falls. AP is stimulated by the absence of TH to produce an excess of TSH. Thyroid hyperplasia results, but in this case with no uptake of iodine.

easy and ready uptake of iodine by the thyroid. It appears then that at least one action of this drug on the function of the thyroid is to interfere with the iodinization of the thyroglobulin molecule, thus preventing the elaboration of any physiologically active hormone.

SUMMARY

Hyperplasia of the thyroid was produced in one group of rats by the administration of thiocyanate, in another by thiouracil. The thiouracil goiters were the larger. Tracer doses of radioactive iodine were administered to the control and treated animals four hours before they were sacrificed.

The average uptake of radioactive iodine by the control rat thy-

roids was 56% of the administered tracer dose, that by thyroids of thiocyanate treated rats, 87%, and that by thiouracil treated, 10%.

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THE EFFECT OF SMALL DOSES OF DIETHYLSTILBESTROL ON THE ANTERIOR HYPOPHYSIS OF THE IMMATURE RAT

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INVESTIGATORS are not agreed whether the secretory activity of the anterior hypophysis is stimulated or depressed by stilbestrol and other estrogens. Diethylstilbestrol has been shown to alter significantly both reproductive activity (Noble, 1938) and carbohydrate metabolism (Janes and Nelson, 1942; Fry, Miller and Long, 1942) through the mediation of this gland. In view of this evidence and the widespread clinical use of stilbestrol, exact information concerning the possible stimulating or inhibiting effect of stilbestrol on each of the hypophyseal cell types is desirable. Morrell and Hart (1941), Von Haam, Hammel, Rardin and Schoene (1941) and Foster (1942) have demonstrated that diethylstilbestrol will simulate the naturally occurring estrogens in bringing about a reduction in number of basophiles and acidophiles and an increase in the relative number of chromophobes. If this substance possessed the capacity to cause either basophiles or acidophiles to increase in number, such a response would have been masked by the degranulation induced by the large doses, or prolonged treatments, which have been employed in most previous experiments. Therefore, we have undertaken a study of the cellular changes in the anterior hypophysis brought about by treatment with diethylstilbestrol in doses close to the minimal effective level, administered over brief periods of time. These findings are being correlated with those in the ovaries and other endocrine glands but only the structural alterations in the pituitary gland will be described in this communication. The results of our study show that the acidophiles are stimulated by diethylstilbestrol.

MATERIALS AND METHODS

Daily subcutaneous injections of diethylstilbestrol[1] in doses ranging from .05 to 4 micrograms (μg.) were administered for 4, 7 or 14 days to 25-day-old female rats of the Long-Evans strain (table 1). Litter-mate controls were kept isolated from the estrogen-treated rats and received comparable amounts

Received for publication January 26, 1944.

[1] We wish to thank Dr. Erwin W. Schwenk of the Schering Corporation, Bloomfield, New Jersey, for supplying us with generous quantities of diethylstilbestrol.

of sesame oil, which was used as solvent for the hormone. In order to minimize the effect of possible errors in mixing the solutions of stilbestrol, new preparations were made repeatedly throughout the course of the investigation. The animals were sacrificed approximately 24 hours after the last injection. The uteri were pressed between pieces of filter paper to remove intra-uterine fluid and weighed as a check on the effectiveness of the treatment. The uteri from the experimental rats showed approximately a fourfold increase in weight over those of the controls. The following groups exhibited somewhat less than average uterine response. In the rats receiving the smallest dose, .05 μg. daily for 7 days, and in those receiving 0.5 μg. for 14 days, the uterine weight was approximately doubled.

The hypophyses of 52 experimental and 23 control rats were fixed in Bouin's fluid and sectioned coronally. Ten 4μ sections from 3 equally spaced zones were preserved for study. After staining with a modified Masson technique, a differential count of the cell types was made on every tenth oil immersion field (× 1125) of the best section from each of the 3 zones. The total number of cells counted in each hypophysis averaged 3244. The mitotic index was determined for each cell type by dividing the total number of mitoses counted in these 3 sections by the total number of oil immersion fields required to cover the sections. An average of 238 fields were examined for mitoses in each hypophysis. The use of serial numbers on the slides concealed the identity of the specimen during these procedures. In addition, the hypophyses of 13 experimental and 5 control rats were fixed in Champy's fluid, osmicated for demonstration of the Golgi apparatus (Nassonov-Kolatschev method) and counterstained by a modification of the Masson technique as described by Severinghaus (1939).

RESULTS

Weight. The hypophysis was noticeably enlarged at autopsy and increased in weight by all doses of stilbestrol employed in this experiment (table 1). The average difference between the mean weights of the hypophyses of the experimental and control rats was about 0.5 mg. Increasing the dosage or duration of treatment did not cause a significant variation from this average difference until 0.5 μg. was injected daily for 7 days. Then the difference was .94 mg. Continuation of this dosage for 14 days did not lead to a further increase in the difference between the weights of experimental and control hypophyses. However, one of the controls in this series, which reached sexual maturity and experienced sexual cycles during the course of the experiment, possessed a hypophysis so much heavier than those of the other controls, that the average for the group was elevated considerably. These increases in weight may not seem great but the consistency with which they were obtained was remarkable. If the exceptional control animal cited above is excluded, the hypophysis of every experimental animal weighed more than that of its litter-mate control.

Acidophiles. Treatment at all dose levels for 4 days led to a relative increase in the percentage of acidophiles with the maximum ef-

fect being induced by 0.5 μg. and 2.0 μg. per day for 4 days (table 2). In these groups the difference between the relative percentage in controls and experimentals was about 15, which represents approximately a 50% increase in the relative number of acidophiles. Although somewhat less marked, the increase was still apparent after 7 days of treatment, a daily dose of only .05 μg. being effective over this period. The injection of 0.5 μg. daily for 14 days led to a slight reduction in

TABLE 1. EFFECT OF DIETHYLSTILBESTROL ON HYPOPHYSEAL WEIGHT AND MITOTIC ACTIVITY

Daily Dose		No. Rats	Mean Wt. (mg.)	Mean Mitotic Index		
				Acidophiles	Basophiles	Chromophobes
4 day treatment						
0.25 μg.	E	7	2.3 ± .2[1]	.148 ± .058	.001 ± .001	.071 ± .031
	C	4	1.7 ± .22	.098 ± .041	.009 ± .01	.072 ± .043
0.5 μg.	E	9	2.2 ± .47	.194 ± .062	.005 ± .009	.073 ± .05
	C	5	1.7 ± .21	.091 ± .038	.008 ± .009	.075 ± .041
2.0 μg.	E	6	2.25 ± .27	.233 ± .28	.003 ± .003	.051 ± .029
	C	4	1.7 ± .4	.105 ± .046	.004 ± .004	.062 ± .022
4.0 μg.	E	7	2.7 ± .32	.092 ± .047	.002 ± .002	.033 ± .019
	C	4	2.0 ± .12	.071 ± .015	.005 ± .003	.052 ± .02
7 day treatment						
0.05 μg.	E	6	2.75 ± .56	.126 ± .03	.002 ± .002	.056 ± .006
	C	4	2.2 ± .34	.044 ± .005	.002 ± .003	.045 ± .022
0.25 μg.	E	5	2.84 ± .63	.117 ± .064	.003 ± .001	.056 ± .043
	C	3	2.36 ± .66	.079 ± .032	.007 ± .002	.059 ± .018
0.5 μg.	E	4	3.28 ± .53	.107 ± .037	.002 ± .001	.067 ± .066
	C	4	2.34 ± .61	.076 ± .028	.005 ± .003	.051 ± .018
14 day treatment						
0.5 μg.	E	5	3.9 ± .21	.194 ± .138	.001 ± .001	.058 ± .036
	C	4	3.3 ± .53	.049 ± .014	.001 ± .002	.035 ± .022

[1] Standard deviation.
E = experimental animals. C = control animals.

the percentage of acidophiles. The effect on the absolute number of acidophiles per field (table 2) paralleled the changes in relative percentage except in the animals receiving daily doses of .25 μg. and .5 μg. for 7 days where an increase of only about 2 acidophiles per field was observed.

Correlated with these changes was an increase in the number of acidophiles in division (table 1). This effect was apparent at all dose levels, being least pronounced at a dose of 4 μg. for 4 days. One experimental animal of the 2.0 μg. group showed a mitotic index of .847, the highest observed. Exclusion of this animal would have given a mean

for this group of .110 which is only a slight increase over the mean of .105 for the controls. Thus, in this series of 4 day injections, lower doses (.25 μg. and .5 μg.) caused a more consistent and regular stimulation of mitotic division in acidophiles than did the higher doses. Injection of .05 μg. per day for 7 days and of 0.5 μg. for 14 days resulted in the greatest apparent stimulation, there being about 3 to 4

TABLE 2. EFFECT OF DIETHYLSTILBESTROL ON DISTRIBUTION OF THE CELL TYPES

Daily Dose		Mean Number per Field			Mean Relative Percentage		
		Acidophiles	Basophiles	Chromophobes	Acidophiles	Basophiles	Chromophobes
4 day treatment							
.25 μg.	E	42.9	13.9	73.0	33.1±5.8[1]	12.2±2.8	56.0±4.0
	C	32.5	18.2	84.6	24.7±1.5	12.7±2.7	62.5±2.1
.5 μg.	E	48.9	12.5	63.4	39.0±6.8	10.0±2.7	51.0±4.9
	C	33.1	16.9	87.3	24.0±2.0	12.5±2.6	64.0±2.2
2.0 μg.	E	49.7	7.4	51.9	45.5±7.2	6.8±1.4	47.5±6.2
	C	38.3	13.4	71.8	30.7±2.1	10.7±1.4	58.0±2.4
4.0 μg.	E	47.3	10.2	54.1	42.1±4.5	8.8±2.7	48.7±4.0
	C	38.8	16.5	74.6	29.7±3.3	12.5±3.5	57.2±2.7
7 day treatment							
.05 μg.	E	47.3	14.5	72.0	35.3±1.5	10.8±1.7	53.8±2.9
	C	36.4	16.2	76.8	27.7±1.2	12.2±2.5	59.5±2.1
.25 μg.	E	44.3	12.9	73.0	33.8±3.6	10.0±0.6	56.0±5.0
	C	42.5	18.3	94.4	27.3±3.6	12.0±0.0	64.0±4.9
.5 μg.	E	44.6	9.9	60.2	38.5±8.5	8.0±1.4	53.5±7.2
	C	42.0	17.2	86.5	27.5±3.1	11.5±0.9	61.0±3.2
14 day treatment							
.5 μg.	E	48.0	6.2	82.0	35.0±4.5	4.6±1.2	60.2±4.8
	C	51.8	10.5	76.4	37.5±3.9	7.5±2.7	54.7±4.9

[1] Standard deviation.

times as many acidophiles in division in the experimentals as in the controls. It should be noted that in these controls, the mean mitotic index was somewhat lower than in any other control groups. Although a few rather fully-granulated acidophiles were seen in division the vast majority of mitotic figures occurred in cells containing only a few brightly stained granules.

Addition of the means of the number of acidophiles, basophiles and chromophobes counted per field (table 2) shows that the total mean number of cells counted per field was reduced by all doses except the lowest, .05 μg. and 0.5 μg. administered for 14 days. For the 4-day injection period, the differences between the total means of the control and experimental groups increased in direct relation to the dosages, these being 5.5, 12.5, 14.5 and 18.3 for daily doses of .25, 0.5,

2.0 and 4.0 μg., respectively. For the 7-day treatment, the differences were 25 and 32 at daily doses of .25 and 0.5 μg., respectively. These observations are, of course, indicative of a progressive cellular enlargement which in the 4-day groups occurred primarily among the acidophiles. Evidence of this effect is readily seen by comparing figures 1 and 4. After 7 days many chromophobes also were hypertrophied.

Cytologically, the changes in the acidophiles were significant and seemed to be most marked in the animals treated for 4 days with doses ranging from .5 μg. to 4.0 μg. Although the hypophyses from these animals contained some acidophiles packed with granules, the majority were hypertrophied cells which tended toward a more spherical form and contained an enlarged and eccentrically-placed nucleus. Although no measurements were made, the nucleoli of some acidophiles seemed to be larger; at least, there were more acidophiles with large nucleoli after treatment with the hormone (compare figures 1 and 4). The acidophilic granules were discrete, brightly stained and most numerous at the periphery of the cell (fig. 4). In the Bouin-fixed preparations the negative image of the Golgi apparatus was enlarged markedly over that of the granule-filled acidophiles (fig. 4). Post-osmication of Champy-fixed hypophyses confirmed this observation and showed the form of the Golgi body to be a large, loose meshwork which extended throughout much of the cytoplasmic space (fig. 3). In the control glands, numerous acidophiles were small, narrow, elongated or stellate cells which contained a small Golgi body and few granules (fig. 1). In most hypophyses which showed extensive stimulation following treatment with stilbestrol, these small cells appeared to have been reduced in number and, probably, converted into the enlarged acidophiles described above.

Basophiles. The injection of .25 μg. of stilbestrol per day for 4 days did not result in a significant change in the relative number of basophiles although the number per field was reduced (table 2). In-

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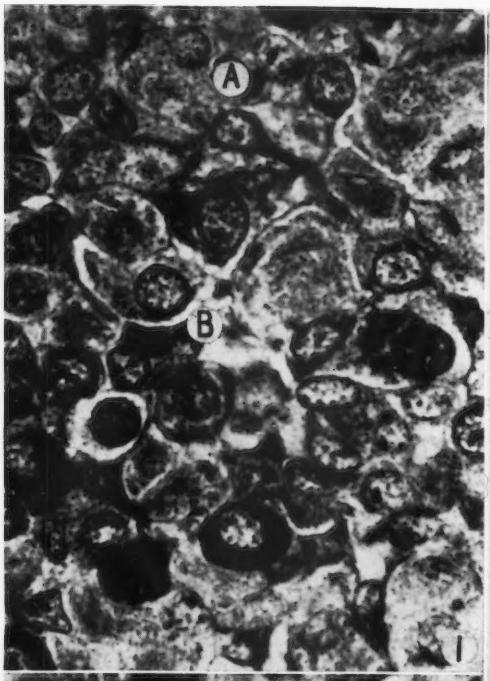
FIG. 1. A 30-day control hypophysis. Well granulated basophiles at either side of B. Narrow elongated acidophile below A. Other larger acidophiles in lower part of field (dark cells). Bouin 3μ Masson ×1160.

FIG. 2. Compact Golgi apparatus in granule-filled acidophile from 30 day hypophysis (litter-mate control for fig. 3). Nassonov-Kolatschev 3μ Altman-Masson ×2800.

FIG. 3. Enlarged Golgi apparatus in acidophile from rat which had received 4 μg. daily for 4 days. Note vesicles and fine meshwork. (Outlines of cell and nucleus in this and fig. 2 drawn with camera lucida.) Nassonov-Kolatschev 3μ Altman-Masson ×2800.

FIG. 4. After daily injection of 2 μg. stilbestrol for 4 days. At right of A is an acidophile with enlarged Golgi region above nucleus. Acidophilic granules (black) are concentrated in upper end of the cell next to the sinusoid and a few are scattered through the Golgi region. Acidophilic granules may be seen in most of the cells in this field. No basophiles visible. Contrast large size of cells and nucleoli to size of those in the hypophysis of the litter-mate control (fig. 1). Bouin 3μ Masson ×1160.

FIG. 5. A 30-day control hypophysis showing large, fully granulated basophiles, one of which is in mitosis. The acidophiles are small and somewhat stellate in shape. Bouin 4μ Masson ×1040.



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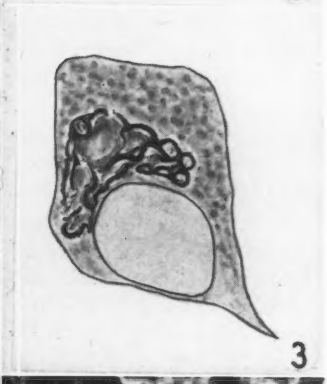

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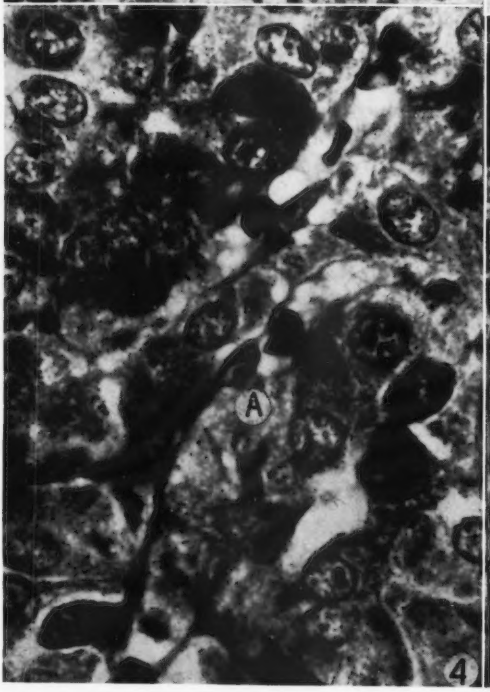

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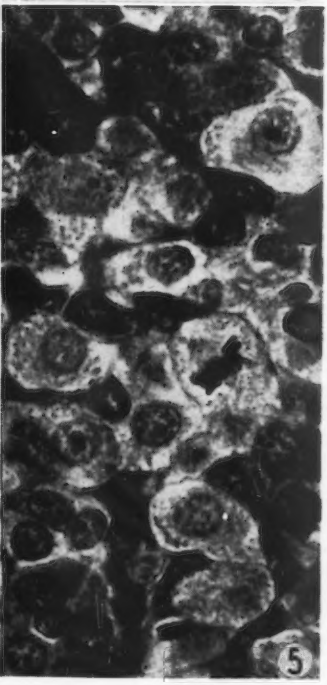

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creasing the amount of hormone injected over this period caused a greater reduction in both the relative and absolute number of basophiles. Continuation of the lower doses for 7 days intensified this effect only slightly. A few mitotic figures were observed in the basophiles of normal animals (fig. 5). If the treatment exerted any effect on their frequency, it was in the direction of a reduction rather than an increase (table 1).

Cytologically, the alterations in the basophiles were more noticeable than indicated by the cell counts. Even though in some cases no reduction in number was observed, many basophiles were, at least, partially degranulated. In some individuals which had received the higher doses, this degranulation was extreme. It was easy to follow these cells through the process of degranulation in the Bouin-fixed preparations, since the nucleus retained its affinity for aniline blue until or after all granules had disappeared. At this time, most of these cells were reduced in size and, exclusive of the condensed cytoplasm of the Golgi region, the cytoplasmic space was generally rather empty.

Chromophobes. A reduction in both the number per field and relative percentage of chromophobes (table 2), occurred coincidentally with the increase in number of acidophiles, this holding true for all doses injected for 4 or 7 days. Conversely, an increase in the number of chromophobes accompanied the reduction in acidophiles and basophiles induced by treatment for 14 days. The changes in mitotic activity among the chromophobes bore a direct relation to these observations. Thus, during the 4-day period when the acidophiles were markedly increased and chromophobes reduced, the mitotic activity of the latter cells was not affected or, at higher doses of 2.0 and 4.0 μg., possibly inhibited (table 1). Continuation of the treatment for 7 days resulted in some stimulation of mitotic activity at 2 of the 3 dosages used. After 14 days, when acidophiles were reduced in number and chromophobes increased, the number of mitoses observed in the chromophobes of the experimental rats was considerably greater than the number in the controls. The 4-day treatment did not induce a marked general enlargement of the chromophobes.

DISCUSSION

Since varied cytological criteria have been employed by other workers in the identification of the pituitary chromophiles, it is necessary to point out, that in our differential counting, no cell was considered an acidophile or a basophile unless it contained differentially-stained granules. Severinghaus (1938) has reported the Golgi apparatus to be entirely dissimilar in form and location in the two chromophilic cell types. However, this organelle would not serve as a satisfactory mark of distinction in the present study since it has been demonstrated that the Golgi apparatus of some acidophiles may

resemble that of the basophiles both normally in the immature rat (Ayers, 1941; Wolfe and Brown, 1942) and after treatment of rats with estrogens (Severinghaus, 1939; Wolfe and Brown, 1942). Numerous workers have confused mitochondria with acidophilic granules. Therefore, we selected Bouin's fluid as a general fixative which destroys mitochondria because of the acetic acid which it contains. In fact, this may explain why our normal acidophile count for 30-day old rats is somewhat lower than that reported by Wolfe (1935), who used a technique which preserved but did not differentiate mitochondria from acidophilic granules. His mean relative percentages for 27–35 day-old rats were: acidophiles, 36.7; basophiles, 8.6; chromophobes, 54.8 as contrasted to our count for these cells of $27 \pm$s.d. 3.9, $12.3 \pm$s.d. 2.9 and $60.5 \pm$s.d. 4.2, respectively.

At present, it is not clear whether shifts in the cell population of the anterior hypophysis occur by conversion of one type of cell into another, by mitotic division of certain cells, or by a combination of both factors. In the opinion of Severinghaus (1938) "The scarcity of mitotic divisions rules out the possibility that specific cells multiply sufficiently to change the relative cell proportions. They must then change from one specific cell type into another." However, Pomerat (1941) recently used the colchicine technique to demonstrate that accelerated mitotic division of basophiles is adequate to explain their increased number following castration. This forces one to consider the rôle played by mitosis in the augmentation of the acidophiles induced by stilbestrol. Even without the use of a substance which arrests mitoses, we found that in all groups studied (table 1) the mitotic activity of the acidophiles was increased and, in one group, quadrupled. It has been demonstrated by Lewis and Lewis (1917) that about 2–3 hours are required for mitotic division of mesenchyme cells grown in tissue culture. If this evidence can be taken as an indication of the time consumed in mitosis by cells of the hypophysis, the small increase in mitotic figures in acidophiles after treatment with 4 μg. for 4 days may not seem surprising, since variations in the rate of absorption of the hormone might have led to a low ebb of stimulation at the time of autopsy. On the other hand, it may be possible that lower doses are more effective in stimulating acidophiles to divide. Consideration of this time element also emphasizes the significance of the increased number of mitoses observed in acidophiles in this experiment. Until it is possible to present concrete evidence for the conversion of chromophobes to acidophiles following injections of stilbestrol, it must be inferred that accelerated mitotic activity in the acidophiles is the primary explanation for their increased number in immature rats subjected to such treatment. Similar stimulation of the acidophiles to mitotic division has been reported in the rat following treatment with naturally occurring estrogen (Wolfe and Chadwick, 1936) and in the monkey, following treatment with

estrogen plus androgen (Dawson, 1942). The acceleration of mitotic activity in acidophiles but not chromophobes by treatment for 4 days, and the increase in number of mitoses among chromophobes as the treatment was prolonged, suggests the probability that these latter chromophobes had been derived from the acidophile line through degranulation. This inference is supported further by the reduction in percentage of acidophiles after 14 days of treatment in spite of the high mitotic index for these cells. There would seem to be little question that chromophiles are converted to chromophobes through degranulation. Indeed, the trend of events observed in this study may give a clue to the origin of the hypertrophied chromophobes with large Golgi bodies exhibited by the hypophyses from rats receiving heavy estrogen treatment (Severinghaus, 1939). Unless one wishes to postulate that the point of stimulation is shifted to include basophiles at dosage levels higher than those we employed, it would seem possible that these chromophobes are derived from acidophiles.

The full significance of the increase in the relative percentage and number of acidophiles per field can be grasped only when viewed in the light of the contemporaneous enlargement of the gland as a whole. Thus, the magnitude of the stimulation on the total acidophile population of the hypophysis is far greater than appears from the counts alone. Only one other report of a similar nature could be found in the literature. Wolfe and Chadwick (1936) injected 10 or 200 R.U. of estrin per day for 5 or 10 days into immature female rats and observed an increase in the absolute number of acidophiles, *i.e.*, number per section, but the greatest increase in average percentage of acidophiles was only from 35.2% to 37.2% and this change followed use of the lower dosages. Finerty (personal communication) injected .055 μg. of estradiol per day for 10 days into 31 day-old rats and found the average percentage of acidophiles to be 29.3% as contrasted to 23.7% for ovariectomized controls. The increases in percentage of acidophiles reported by these investigators are considerably less than those observed in our material following treatment with a synthetic estrogen.

Important correlations may be made between the effects of estrogen on the structure and on the function of the hypophysis. Some evidence suggests that the acidophiles produce both luteinizing hormone (LH) (Severinghaus, 1937) and lactogen (Schooley and Riddle, 1938; Azimov and Altman, 1938). A close relationship between these two principles may be indicated by the fact that both stimulate corpora lutea, LH being understood to induce their formation and lactogen having been reported to maintain the function of these structures (Evans, *et al.*, 1941; Cutuly, 1942). The naturally occurring estrogens have been shown to induce the release of LH (for review of evidence see Fevold, 1939) and lactogen (Meites and Turner, 1942a) from the hypophysis. Furthermore, these substances (Meites

and Turner, 1942c) and diethylstilbestrol (Meites and Turner, 1942b) increase the lactogenic content of the hypophysis. Possible similarity of action of the male sex hormone is indicated by the capacity of testosterone also to induce an increase in the amount of lactogen in the rat hypophysis (Meites and Turner, 1942b), to increase the reduced number of acidophiles in the hypophysis of senile male rats (Wolfe, 1941), and to increase the number of acidophiles in normal male rats (Wainman, Reese, and Koneff, 1942).

The evidence in support of the stimulative action of estrogens on the acidophiles may be summarized as follows: injection of estrogens leads to (1) increased mitotic activity in acidophiles, (2) increase in number of acidophiles, (3) hypertrophy of the Golgi apparatus in acidophiles which still contain specific granulation, (4) liberation of hypophyseal factors, the production of which on the basis of other evidence can be related to the acidophiles, and (5) castration results in cytological regression of acidophiles (Severinghaus, 1938).

Desclin (1935) and Severinghaus (1937) have reported an increase in the number of basophiles in the rat following treatment with naturally occurring estrogens. One must hasten to point out that Desclin described acidophilic granules in the cells which he called basophiles. The admission of such a condition would be disastrous to the currently-held concept that basophiles and acidophiles represent independent cell lines. Severinghaus based his identification chiefly on the appearance of the Golgi apparatus and mitochondria. Nelson (1934), employing similar criteria, reported many of the chromophobes in hypophyses from estrogenized rats to be in reality degranulated basophiles. Nevertheless, he observed that physiological evidence points to an inhibition of the basophiles. In our counts such degranulated cells would appear as chromophobes. We have not found evidence of an increase in number or mitotic activity of basophiles under the conditions of our experiment. It is difficult to recognize a small basophile when in division but these cells could have been confused only with the chromophobes among which there was no increase in number or mitotic activity except in some groups after prolonged treatment. Contrary to our observations, Wolfe and Chadwick (1936) failed to find any basophiles in mitotic division in normal immature rats. The identification of most of these cells in our study could not be disputed because of their unmistakable granulation (fig. 5).

Our results bear directly on the problem of the threshold levels at which the basophiles and acidophiles may be affected by the concentration of estrogenic hormone in the blood. It has been stated (Severinghaus, 1937) that the acidophiles are activated by estrone at a dosage level above that necessary to degranulate the basophiles. In contrast, we found that diethylstilbestrol induced an increase in number and mitotic activity of the acidophiles at a level too low to reduce

significantly the relative number of basophiles. Some of the basophiles exhibited definite degranulation under this treatment but it was not possible, in these experiments, to show that this change preceded those observed in the acidophiles.

SUMMARY

The daily injection of diethylstilbestrol in doses ranging from .05 to 4 μg. for 4 or 7 days into 25 day-old female rats caused the following changes in the hypophysis: (1) increase in weight of the gland, (2) increased mitotic activity in the acidophiles with increase in their relative and absolute number, (3) hypertrophy of the Golgi apparatus in the acidophiles, (4) degranulation and reduction in number of the basophiles with no effect on their mitotic activity and (5) reduction in the relative and absolute number of chromophobes with little change in their mitotic activity. Continuation of injections of 0.5 μg. daily for 14 days resulted in a slight reduction in number of acidophiles, increase in number of chromophobes and an increase in the mitotic activity of both these of cell types. It was concluded that diethylstilbestrol exerts a stimulative action on the acidophiles.

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THE EFFECT OF THIOURACIL ON THE FORMATION OF THYROXINE AND DIIODOTYROSINE BY THE THYROID GLAND OF THE RAT WITH RADIOACTIVE IODINE AS INDICATOR[1]

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IT HAS BEEN repeatedly shown during the last few years that the administration of thiourea and of a number of its derivatives produces hyperplasia and hypertrophy of the thyroid gland (MacKenzie and MacKenzie, 1943; Astwood, Sullivan, Bissell and Tyslowitz, 1943; Astwood, 1943; Kennedy, 1942; Richter and Clisby, 1942). The evidence obtained in the intact animal indicates that these compounds depress the activity of the thyroid gland (MacKenzie and MacKenzie, 1943; Astwood, Sullivan, Bissell and Tyslowitz, 1943).

Recent investigations in this laboratory have demonstrated that these compounds inhibit the conversion of inorganic iodide to thyroxine and diiodotyrosine by the thyroid gland (Franklin and Chaikoff, in press). This was shown by an *in vitro* procedure in which surviving thyroid slices were incubated in a Ringer's medium containing radioactive inorganic iodide. The addition of thiourea, allylthiourea and thiouracil strongly retarded the formation of thyroxine and diiodotyrosine. Allylthiourea markedly depressed this formation at a concentration as low as 10^{-4} M.

The present investigation deals with the influence of one of these compounds on the formation of thyroxine and diiodotyrosine by the intact thyroid gland. It is shown that the feeding of thiouracil to the rat interferes with the incorporation of injected radioactive iodine into thyroxine and diiodotyrosine by its thyroid gland.

METHODS

Six rats weighing from 270–305 gm. were fed *ad libitum* a diet[2] containing 0.035 per cent thiouracil for 7 days. Each animal ingested from 3.4–4.2 mg. of thiouracil per day. During the subsequent period of observation, these rats

Received for publication January 27, 1944.
[1] Aided by grants from the Commonwealth Fund and the Committee for Research in Endocrinology of the National Research Council.
[2] The diet fed consisted of 37 per cent casein, 34 per cent whole wheat, 8 per cent whole milk powder, 5 per cent fish meal, 5 per cent alfalfa, 2.5 per cent lard, 2.5 per cent fish oil, 5.3 per cent NaCl, and 0.7 per cent $CaCO_3$.

were fed the same diet but with no thiouracil. They were then divided into 3 groups of 2 rats each and injected with radioactive inorganic iodide on the 0, 7th and 14th day after the thiouracil feedings had been discontinued. The thyroid glands of the animals were excised 48 hours after the introduction of the radioiodide and these glands analyzed for total radioactive iodine and for radiothyroxine and radiodiiodotryosine. Two rats served as controls. These are designated normal in fig. 1. These animals received the diet with-

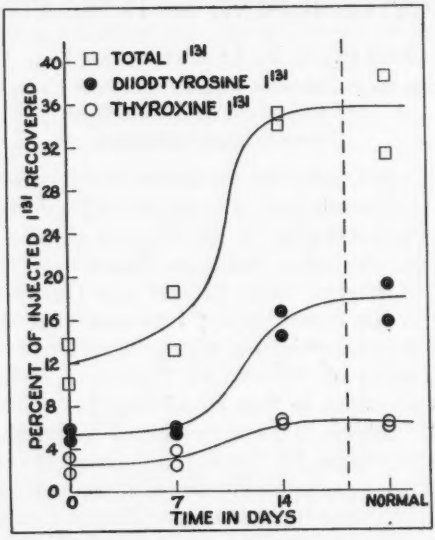

FIG. 1. The uptake of I^{131} by thyroid glands of normal and thiouracil-treated rats 48 hours after its intraperitoneal injection.

All rats except the normal were fed thiouracil for 7 days. The time in days refers to the interval between the last feeding of thiouracil and the time of injection of the I^{131}.

out thiouracil for 7 days before they were injected with the radioactive iodide.

Radioactive inorganic iodide containing no carrier was used in this investigation. Its preparation has been described elsewhere (Perlman, Chaikoff and Morton, 1941). Each rat received intraperitoneally 3 microcuries of I^{131} in 1.0 cc. of isotonic NaCl.

The animals were anaesthetized with nembutal before excision of the thyroid glands. The glands of each animal were placed in an Erlenmeyer flask containing 6.0 cc. 2N NaOH and hydrolyzed for 8 hours on the steam bath. The radioactive thyroxine, radioactive diiodotyrosine and inorganic I^{131} were separated and determined as described elsewhere (Perlman, Morton and Chaikoff, 1941).

The thyroid glands of the normal rats weighed 40 and 50 mg.; those that were removed from animals injected on the 0, 7th and 14th day after the feeding of thiouracil had been discontinued weighed respectively 100 and 70 mg., 60 and 50 mg., and 60 and 40 mg.

RESULTS

Uptake of total I^{131} by thyroid glands. The uptake of I^{131} by thyroid glands of normal and thiouracil-treated rats is recorded in fig. 1. Forty-eight hours after its injection the thyroid glands of the normal rats (i.e. those that received no thiouracil) contained 31–38 per cent of the I^{131}. The rats that were injected with radioactive iodine on the same day that they received their last meal containing thiouracil accumulated 10–14 per cent of the injected I^{131}. Two rats were injected with I^{131} on the 7th day after the feeding of thiouracil was discontinued; 13–19 per cent of the injected I^{131} was recovered in their thyroid glands. Thirty-four to thirty-five per cent of the injected I^{131} was recovered in the thyroid glands of the rats in which 14 days were allowed to elapse between the time of the I^{131} injection and the last feeding of thiouracil.

Recovery of thyroxine I^{131} and diiodotyrosine I^{131} in thyroid glands. Six per cent of the injected I^{131} was converted to thyroxine in the glands of the normal rats. Less of the I^{131} was recovered as thyroxine in the glands of 2 groups of rats: 1) those that received radioiodide at the end of the 7-day feeding with thiouracil and 2) those that were injected with I^{131} 7 days after the administration of thiouracil had been stopped. In these 2 groups of rats from 1.7–3.8 per cent of the injected I^{131} was recovered in the form of thyroxine. When the interval between the last ingestion of thiouracil and the injection of radioiodine was extended to two weeks, the recovery of I^{131} as thyroxine was similar to that observed in normal animals.

The glands of the normal rats contained 16–19 per cent of the injected I^{131} as diiodotyrosine. Similar amounts were recovered in this form in the glands of the rats that had been deprived of thiouracil for 2 weeks. Reduced amounts, however, were observed in the 2 other groups of rats.

DISCUSSION

These findings show that the daily feeding of 3.4–4.2 mg. of thiouracil for 7 days depresses the uptake of injected I^{131} by the thyroid gland of the rat. This effect of thiouracil did not remain for long after its feeding was stopped. Under the conditions of this study, the recovery of the gland, as judged by its capacity to remove injected radioiodine, was complete in 2 weeks. These changes in the I^{131}-concentrating capacity of the thyroid tissue were reflected in the amounts of radiothyroxine and radiodiiodotyrosine found in the glands. The thyroids obtained from rats injected with radioiodide on the last day of thiouracil feeding as well as those removed from rats injected 7 days after its feeding had been stopped contained about half the amounts of radiothyroxine and about one-third the amounts of radiodiiodotyrosine found in the normal glands.

No I^{127} was used as carrier in the preparation of the radioactive

iodine. Such samples of radioactive iodide contain only insignificant amounts of iodine, hence their injection does not increase the amount of iodine already present in the animal. Since the I^{131} introduced is in the form of inorganic iodide, it serves, as soon as mixing occurs, to label plasma's inorganic iodide and that portion of the inorganic iodide in tissues that comes into rapid equilibrium with that of plasma. The absolute amounts of I^{127} labeled in this way are not known; no information is therefore available on the absolute quantity of I^{127} represented by recovery of a given amount of radioactivity. In each animal, however, the fate of a particular portion of radioactive iodine represents the fate of a certain fraction of the *initially labeled total circulating iodide*. If the inorganic iodide content of plasma of the thiouracil-treated rats differs little from that of the normal rats, then the values recorded in fig. 1 show that in the rats that received thiouracil the I^{127} uptake by the gland is depressed and that less of the initially labeled iodide has been converted to thyroxine and diiodotyrosine.

SUMMARY

The effect of thiouracil on the uptake of injected radioactive inorganic iodide (I^{131}) by the thyroid gland and its conversion in this tissue to thyroxine and diiodotyrosine was determined.

1. Rats weighing 270–305 gm. were fed 3.4–4.2 mg. of thiouracil daily for 7 days. They were injected with I^{131} 0, 7 and 14 days after the administration of thiouracil had been discontinued. The animals were killed 48 hours after the injection of the I^{131} and the content of total I^{131}, thyroxine I^{131} and diiodotyrosine I^{131} of their thyroid glands measured.

2. At the end of the 7-day period of thiouracil feeding, the capacity of the gland to concentrate injected I^{131} and the amounts of I^{131} converted in the gland to thyroxine and diiodotyrosine were depressed.

3. Both the I^{131}-concentrating capacity of the gland and the amounts of radiothyroxine and radiodiiodotyrosine formed in the thyroid were restored to normal 2 weeks after the administration of thiouracil had been discontinued.

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THE ESTROGEN-PROGESTERONE INDUCTION OF SEXUAL RECEPTIVITY IN THE SPAYED FEMALE MOUSE[1]

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ESTROGEN followed after a suitable interval by a small amount of progesterone is more effective than estrogen alone in inducing mating responses in the spayed female guinea pig (Dempsey, Hertz and Young, 1936) and in the spayed female rat (Boling and Blandau, 1939). Heat could be induced consistently in the spayed females of these species only when the two hormones were injected in proper sequence. The estrous behavior following estrogen-progesterone administration more closely resembled normal estrus in character and duration than did that following the injection of estrogen alone. These investigators therefore concluded that in the normal female guinea pig and rat sexual receptivity is the result of a conditioning action of follicular estrogen followed by the action of progesterone secreted by the preovulatory follicle. The literature supporting this view has been extensively reviewed by Young (1941).

Since the guinea pig and rat have been considered unique in possessing this mechanism of heat induction, it seemed advisable to extend this work to another species. This paper presents the results of experiments which indicate that the estrogen-progesterone mechanism controls the induction of sexual receptivity in the mouse.

MATERIALS AND METHODS

One hundred and fifty spayed mice of several genetic strains were employed in the experiments. No strain differences in threshold or quality of response to hormonal treatment were apparent. Each ovary was removed with its capsule intact along with part of the ovarian fat body, the Fallopian tube, and a portion of the uterine horn. No evidence of ovarian regeneration was seen at autopsy.

The hormones estradiol benzoate and progesterone,[3] dissolved in sesame

Received for publication February 1, 1944.

[1] This investigation was supported in part by a grant from the National Research Council, Committee for Research in Problems of Sex; grant administered by Dr. R. J. Blandau.

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[3] Progynon-B stated to contain 10,000 R.U. per 1.66 mg. and Proluton, kindly furnished by Dr. E. Schwenk of the Schering Corporation.

oil, were injected subcutaneously on the belly or on the flank. The animals were allowed to rest for at least ten days between experiments. During this time the subcutaneous oil cysts, which frequently formed, were ruptured manually and the site massaged to promote absorption of the oil.

Two procedures were employed in testing for heat. In the first series of experiments one or two females were placed with twice the number of vigorous males and allowed to remain overnight. On the following morning the females were examined for the presence of a copulation plug. During later experiments the animals were observed continuously throughout the mating trial period. The criterion of mating in the case of animals observed continuously was the occurrence of three or four copulations. Results obtained with the copulation plug and constant observation methods were in good agreement.

Because of the difficulty of making continuous observations during several successive days, it was found necessary to test the mating responses of a given group of animals during one period of approximately 18 hours. This mating trial period included the second or the third night following the initial injection of estradiol. Preliminary trials showed that only a very small percentage of the spayed females would mate before 48 hours following the injection of estradiol. This procedure of testing for heat on one night only was checked by a series of mating trials during the periods approximately 18 hours in duration beginning 24, 48, 72 and 96 hours after the injection of 5, 10 and 100 R.U. of estradiol. The copulation plug method was employed for these extended observations. In almost all instances the beginning of heat occurred later than 48 hours after the injection of estrogen. Almost all of the animals destined to come into heat from a given injection of estrogen had commenced mating before 96 hours after the injection. With large amounts of estradiol mating sometimes continued irregularly for several days.

To serve as normal controls and to ensure that the rest period was sufficient for the destruction of residual hormone from the previous injection, small groups of spayed females which had not received injections for 10 or more days were frequently placed with males during the mating trial periods of the injected animals. These control animals never mated.

For a given amount of estrogen a considerably larger percentage of females mated when they were placed with the males each night and removed during the day than when allowed to remain with the males continuously for several days. This appeared to be the result of a failure in male activity rather than a lowered receptivity of the females.

RESULTS

Estrogen-induced mating behavior

Table 1 summarizes the data obtained from mating trials of spayed mice following the injection of estradiol benzoate alone. In no experiment did every animal of a group mate, regardless of the amount of estrogen administered.

None of 13 animals injected with only 1 R.U. of estradiol mated during the period beginning 48 hours after injection. Three of 22 females injected with the same amount of estrogen mated during

the period beginning 72 hours after injection. Apparently a longer period is required for the action of estrogen when small doses are employed. With increasing amounts of estrogen the percentage of animals mating increased to maxima of 73.5% during the period beginning 48 hours after the injection of 10 R.U. and 80% during the period beginning 72 hours after the injection of 100 R.U.

With the exception of the groups receiving 1 R.U. and 100 R.U. injections, a larger percentage of animals mated during the period

TABLE 1. INDUCTION OF MATING IN SPAYED MICE BY INJECTIONS OF ESTRADIOL BENZOATE

Amount of Estradiol Benzoate	Period of Observation Following Injection	Number of Mice Injected	Number of Matings in Injected Mice	% of Injected Mice Mating
R.U.	*hours*			
1	48– 66	13	0	0.0
1	72– 90	22	3	13.6
2	48– 66	20	8	40.0
2	72– 90	22	3	13.6
3	48– 66	27	11	40.8
3	72– 90	42	12	28.6
5	48– 66	22	14	63.7
5	72– 90	46	26	56.5
5	24–114	24	14	58.5
7	72– 90	8	5	62.5
10	48– 66	15	11	73.5
10	72– 90	9	6	66.7
10	24–114	10	6	60.0
100	48– 66	10	5	50.0
100	72– 90	10	8	80.0
100	24–114	10	8	80.0

beginning 48 hours after injection than mated in the 72 hour groups. The average latent period for the induction of heat by estradiol in the spayed mouse was found to be about 60 hours. Therefore, the lower percentage of animals which mated in the period beginning 72 hours after injection can be explained on the basis that the majority of animals came into heat some time after 48 hours, but some of these did not remain in heat until the 72 hour mating trial period. Evidence for this conclusion is seen also in the fact that with higher doses of estrogen (5–10 R.U.) the discrepancy is diminished, more animals remaining in heat beyond the second day following the injection.

Only 5 of a group of 10 animals injected with 100 R.U. of estradiol in a single dose mated during the period beginning 48 hours after the injection. In another group of 10 animals injected similarly but placed with males during the period beginning 72 hours after injection, 8 females mated. In mating trials conducted on a third group of 10 animals during the periods beginning 24, 48, 72, and 96 hours after the injection of 100 R.U. of estradiol, 4 animals mated during the

period beginning at 48 hours, and 4 others did not come into heat until the 72 hour period. No animals in this group mated during the period beginning at 24 hours, and only those already in heat mated during the period beginning 96 hours after the estrogen injection. The basis for the apparent delayed action of large amounts of estrogen is not known.

In order to test the effect of an additional injection of estrogen, 10 spayed mice were injected with 5 R.U. of estradiol and 48 hours later received an additional injection of 10 R.U. The females were then placed with the males. Six of the ten animals mated, approximately the same percentage as that found in the groups receiving 5 R.U. only.

Estrogen-progesterone Synergism in the Induction of Heat

The optimum quantity of progesterone required to induce heat consistently in spayed mice following adequate conditioning with

TABLE 2. INDUCTION OF MATING IN SPAYED MICE BY INJECTIONS OF EXTRADIOL BENZOATE FOLLOWED 48 HOURS LATER BY PROGESTERONE

Amount of Estradiol Benzoate	Amount of Progesterone	Number of Mice Injected	Number of Matings in Injected Mice	% of Injected Mice Mating	Average Latent Period
R.U.	*Mg.*				*hours*
1	0.01	5	0	0.0	—
1	0.03	5	0	0.0	—
1	0.05	19	9	47.3	9.2
1	0.08	5	3	60.0	9.5
2	0.03	5	4	80.0	7.8
2	0.05	19	13	68.5	8.5
2	0.10	9	4	44.5	?
3	0.05	30	24	80.0	7.9
5	0.05	28	26	93.0	8.0
10	0.05	26	26	100.0	7.0
20	0.05	8	8	100.0	7.6
100	0.05	18	18	100.0	7.8

estradiol was found to be about 0.05 mg. The optimum time interval between injections is 48 hours. Table II shows the number and percentage of matings induced by the combined effect of estradiol and progesterone.

Nine of 19 animals receiving only 1 R.U. of estradiol mated following the injection, 48 hours later, of 0.05 mg. of progesterone. This amount of estrogen alone was completely ineffective in 13 mice at 48 hours and was followed by mating of only 3 of 22 mice at 72 hours. The percentage of animals mating following injections of progesterone increased with increasing amounts of estradiol to 100% at 10 R.U. and above. The highest percentage of matings following estrogen injections alone occurred in the groups which receive

100 R.U. This percentage of matings (80%) was obtained with only 3 R.U. of estradiol when the estrogen injection was followed 48 hours later by 0.05 mg. of progesterone.

The average latent period for the action of progesterone in those animals which were observed continuously throughout the mating trial period was 7.9 hours. The range was from less than 5 to 15 hours. The average latent period tended to decrease with increasing dose of estradiol.

The mating responses induced by injections of progesterone in estrogen-conditioned spayed mice appeared to be more normal in character than those observed in the animals receiving estrogen stimulation alone. The criteria of normality were the intensity and the precision of performance of the female sexual pattern, the duration of individual copulations and the consistency of repetition of the mating act throughout the induced estrus period. After the deposition of a vaginal plug the females of both groups (and normal estrous females) often became repellent toward the male. For this reason the duration of heat could not be observed.

DISCUSSION

Several investigators have reported the induction of mating behavior in spayed mice by injections of estrogen alone (Allen and Doisy, 1923; Allen et al., 1924; Wiesner and Mirskaia, 1930; Marrian and Parkes, 1930; Bourg, 1931; and Hemmingsen, 1933). In no case, however, did all of the injected spayed animals mate, regardless of the amount of estrogen administered. In general the percentages of spayed mice mating in response to estrogen injection in the present investigation exceeded those reported by other investigators for equivalent dosages.

Both Wiesner and Mirskaia (1930) and Marrian and Parkes (1930) noted the failure of the vaginal cornification unit as an index of mating activity. The former workers suggested that a factor supplemental to estrogen might be involved in the induction of mating in the mouse.

The results presented above demonstrate the superiority of estrogen followed by progesterone over estrogen alone in the induction of heat in the spayed mouse. The observed greater normality of the estrogen-progesterone heat over that induced by estrogen alone, when considered along with the synergistic action of the hormones, suggests that in the normal mouse, as in the guinea pig and rat, estrus is the result of the action of progesterone in the estrogen-conditioned animal. Ample physiological and morphological evidence for the production of progesterone by the ovarian follicle prior to ovulation (and consequently prior to corpus luteum formation in the strict sense) has been presented and is reviewed elsewhere (Young, 1941; Hisaw and Astwood, 1942).

Qualitatively the demonstration of estrogen-progesterone syner-

gism in the induction of heat in the spayed mouse agrees closely with the results obtained for the spayed guinea pig (Dempsey, Hertz and Young, 1936) and for the spayed rat (Boling and Blandau, 1939). The relative sensitivity to these hormones, however, varies in the different species. Heat was induced in 65% of the guinea pigs injected with 1000 I.U. of estradiol benzoate (600 R.U.) by Dempsey, Hertz and Young (1936). One hundred R.U. of the same hormone induced heat in 90% of spayed rats (Boling and Blandau, 1939) and in 80% of spayed mice (present investigation). However, mating followed injection of as little as 1 R.U. of estradiol in spayed mice in a few cases, whereas Boling and Blandau did not obtain heat in the rat with less than 10 R.U. Mating by 16% of the rats (6 of 38 injections) followed injection of 10 R.U., while from 60 to 73.5% of the mice receiving this amount of estrogen mated. However, 10 R.U. of estradiol benzoate is an adequate conditioning dose in both the rat and the mouse, whereas only 8 R.U. (40 I.U.) of theelin is required in the guinea pig.

In regard to progesterone sensitivity Dempsey, Hertz and Young (1936) found 0.1–0.2 mg. the optimum quantity of progesterone to induce the mating response in suitably estrogen-stimulated guinea pigs. Boling and Blandau (1939) found the optimum quantity of this hormone for the rat to be 0.3–0.4 mg. In the spayed mouse, as shown above, 0.05 mg. of progesterone will consistently induce sexual receptivity when it is injected following adequate estrogen conditioning.

Thus, although the mouse appears to react to the smallest amounts of the two female sex hormones, when the average weights of females of these three species are taken into consideration, it appears that the rat is the most sensitive to estrogen and the guinea pig most sensitive to progesterone. In this connection, it has been shown that the injection of 0.05 mg. of progesterone daily is sufficient to inhibit estrus in the normal guinea pig (Dempsey, 1937), whereas 1.5 mg. per day are required in the rat (Phillips, 1937) and 0.2 mg. daily in the mouse (Robson, 1938).

The latent period between the injection of progesterone and the inception of heat is longer in the mouse than in the rat or guinea pig. The average latent period in the mouse ranged from 7.0 to 9.5 hours, tending to diminish with increasing amounts of estrogen. The latent period in the adequately estrogen-conditioned spayed rat was 2 to 3 hours (Boling and Blandau, 1939), and the average value for the guinea pig (Dempsey, Hertz and Young, 1936) was 4.8 hours following the progesterone injection. The considerably lengthened latent period in the mouse may be due in part, at least, to the fact that the criteria for the inception of estrus are not as sharp in the mouse as in the other two species.

SUMMARY

Estrogen and progesterone administered in sequence are more effective than estrogen alone in inducing sexual receptivity in the spayed female mouse. The optimum amount of progesterone is 0.05 mg., and the optimum time interval between injections is 48 hours. The mating behavior induced by the synergistic action of progesterone and estrogen appears to be more normal in character than that induced by estrogen alone. It is concluded therefore, that the mouse is another of the species in which estrus is the result of a conditioning effect of estrogen followed by the action of progesterone secreted by the ovarian follicle prior to ovulation.

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THE EFFECT OF LIGHT ON MATURATION AND THE ESTROUS CYCLE OF THE COTTON RAT, SIGMODON HISPIDUS HISPIDUS[1]

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IN RECENT years a large number of investigations have shown that reproductive activity in some cold-blooded vertebrates, in many birds and in various mammals is modified by the length of day. A number of reviews of the subject are available (Bissonnette, 1936a; Bissonnette, 1937; Marshall, 1936; Rowan, 1938a; and Rowan, 1938b). Since the research to be presented here deals with a mammalian form, a short review of previous work on mammals is included.

Some mammals, such as the guinea pig (Dempsey, Myers, Young and Jennison, 1934), the rabbit (Smelser, Walton and Whetham, 1934), the hedgehog (Allanson and Deanesly, 1934) and the ground squirrel (Wells, 1935), are apparently not influenced to any marked extent by experimental variation of day length and light intensity. Increased day length in the fall produced estrus in winter in the ferret (Bissonnette, 1932) and caused breeding to be advanced 6 weeks in the raccoon (Bissonnette and Csech, 1937). In the cat, early estrus was induced by increased length of daily illumination during the anestrous period (Dawson, 1941). Litter production was markedly increased in *Microtus* (meadow mouse) when the animals were subjected to artificial 15 hour days (Baker and Ranson, 1932). Increased daily light duration in the fall caused breeding to be advanced 2 months in *Peromyscus* (white-footed mouse) (Whitaker, 1936; Whitaker, 1940). In some animals responses are elicited by shortening the length of day following a period in which the day was lengthened. For example, Bissonnette (1941) found that the breeding cycle of the goat, an animal which normally breeds in the fall, is controlled in such a way that short days induce breeding cycles while long days inhibit them.

Browman (1937) reported a prolonged cornification of the vagina in white rats kept in continuous light. Fiske (1941) found that immature female white rats kept in the light from birth or from the 21st day of life came into sexual maturity, as evidenced by the time of vaginal introitus, somewhat earlier (6 days) then females kept

Received for publication February 2, 1944.
[1] Aided in part by a grant from the Wisconsin Alumni Research Foundation.

under ordinary laboratory conditions and much earlier (16 days) then females kept in darkness. Young females kept in the light exhibited unusually long periods of estrus and slightly lengthened periods of diestrus, while those in the dark had an increased number of days on which they showed metestrous smears. The pituitaries, ovaries and uteri of 77 day old female rats kept in the light for 8 weeks were larger than those of females living under normal laboratory conditions and considerably larger than those of females kept in darkness. The pituitaries, testes and seminal vesicles of males kept in the light for 77 to 250 days were slightly larger than those of animals living under normal laboratory conditions and much larger than those of animals kept in darkness. Fiske attributes her results to the fact that stimulation of the pituitary by light increases the production and release of FSH, and that, conversely, a lack of light favors a decrease in FSH and an increase, in the female only, in LH secretion. The pituitaries of such animals showed marked cytological modifications (Pomerat, 1942).

The purpose of this work was to determine the effect of constant illumination or darkness on the immature cotton rat and on the estrous cycle of the adult cotton rat, *Sigmodon hispidus hispidus*. The cotton rat differs from the ferret and the raccoon in being polyestrous. Whether it differs from the laboratory rabbit, guinea pig and white rat and resembles *Microtus* and *Peromyscus* by having an anestrous season in the wild cannot be definitely stated. It certainly has no anestrous season in the laboratory. It is less nocturnal in habits than *Peromyscus*, resembling *Microtus* in this respect.

EXPERIMENTAL CONDITIONS

The animals used for this study were kept in a windowless room in the sub-basement, aerated by a ventilating system. Because the room is infrequently used the rats were subject to considerably less disturbance than those kept in the vivarium. The animals kept in the light were housed in cages made entirely of ¼ inch mesh screen, 13 inches long by 9 inches wide by 8 inches high, set in galvanized iron trays containing pine shavings. The cages were placed on a table illuminated from above by 100 watt bulbs equipped with reflectors. Measurement of the illumination at cage level by means of a photometer showed the intensity to average approximately 30 foot-candles. The animals in darkness were located, similarly caged, immediately beneath the table. The back of the table was placed flush against a large cupboard and sheets of heavy canvas, hung from the sides and front of the table, converted the space beneath into a light proof "compartment." The cages were set under the table in a large heavy cardboard box, open only to the front, which was placed at some distance from the cupboard. In this way, air circulated beneath the cupboard and into the dark compartment without light being admitted to the animals.

The temperature (26–27° C.) to which the animals in light were exposed varied from 1 to 2° C. higher than that of the dark compartment. Under normal laboratory conditions in our vivarium the room temperature aver-

ages 24° C. The rats were fed and watered in the same manner as those kept in the vivarium (Meyer and Meyer, 1944). However, no hutches or covering material of any sort were furnished and the animals were constantly under the conditions of the experiment except that the rats kept in darkness were exposed to light for a few minutes every other day during feeding and watering.

EFFECT OF CONSTANT LIGHT OR CONSTANT DARKNESS ON THE IMMATURE COTTON RAT

Sixteen rats, 11 females and 5 males from 3 litters, were exposed to constant illumination from birth to the age of 50 days. Sixteen

TABLE 1. EFFECT OF LIGHT ON MATURATION OF THE COTTON RAT

Experimental Conditions	Age at Autopsy (days)	No. of Animals	Sex	Body Wt.	Gonad Wt.	Uterus Wt.	Vagina	Sem. ves. Wt.	Prostate Wt.
				(gm.)	(mg.)	(mg.)		(mg.)	(mg.)
Constant light, from birth*	50	5	♂	87 (81–93)	1217[1] (1097–1295)	—	—	721 (578–968)	339 (268–418)
		11	♀	69 (57–88)	15.0[2] (7.6–20)	206 (102–353)	9 open[7] 2 closed	—	—
Control	50	4	♂	91 (84–100)	1023[3] (925–1095)	—	—	789 (484–997)	314 (235–360)
		6	♀	82 (70–102)	14.5[4] (9.7–20)	218 (168–301)	5 open[8] 1 closed	—	—
Constant dark, from birth**	50	8	♂	90 (70–104)	793[5] (483–1053)	—	—	273 (18–483)	126 (21–210)
		8	♀	71 (57–90)	10.6[6] (4.8–20)	96 (24–228)	3 open[9] 5 closed	—	—

* Mother removed at 10 days.
** Mother of 2 broods removed at 10 days, 2 at 20 days.
[1] The testes of 2 of these animals descended by 20 days, the remainder by 30 days.
[2] The ovaries of 6 of these animals contained corpora lutea.
[3] The testes of one of the controls descended by 20 days, the remainder by 30 days.
[4] The ovaries of 5 of these animals contained corpora lutea.
[5] The testes of one of these animals descended by 20 days, 2 by 30 days, 4 by 40 days and one by 50 days.
[6] The ovaries of 2 of these animals contained corpora lutea.
[7] The vaginas of 2 of these animals opened by 30 days, 2 by 40 days.
[8] The vaginas of 2 of the controls opened by 30 days, 3 by 40 days.
[9] The vaginas of 2 of these animals opened by 40 days.

rats, 8 females and 8 males from 4 litters, were exposed to darkness for the same period of time. The mothers were removed when the young were 10 days old, except in the case of 2 of the litters kept in darkness; the mother was taken from these young when they were 20 days of age. The animals were autopsied at the age of 50 days. The data are presented in table 1.

The average body weight of the males kept in light or darkness did not differ significantly from that of the control males kept under ordinary laboratory conditions. However, both the average body weights of the females raised under light and of those raised in darkness were definitely less than that of the control females.

The gonads of males kept in the light were heavier than those of control males and much heavier than those of animals kept in darkness. The time of descent of the testes of the males exposed to

light was similar to that of the controls but much earlier, on the average, than that of males exposed to darkness. The average weights of the accessory glands of males kept under light did not differ significantly from those of the controls but were nearly three times the average weights of the accessories of males kept in darkness.

The average weights of the gonads and uteri of females exposed to light did not differ significantly from those of the control females but were definitely greater than those of females exposed to darkness. The time of vaginal introitus in the rats kept in the light was slightly later, on the average, than that of the controls but definitely earlier than that of females kept in darkness.

There were no significant differences in the average pituitary weights of the three groups. The thyroids of the experimental animals were slightly heavier than those of the controls. The average adrenal weight of the males in both experimental groups was greater than that of the control males, while the adrenals of the females kept in light were slightly heavier than those of the control females and females kept in darkness. The thymus glands of animals exposed to light weighed less than those of the control rats; those of the animals exposed to darkness were slightly heavier than those of the controls. As there was no essential difference in the weights of these organs they are not included in table 1.

From these results it appears that exposure to constant light, under the experimental conditions, has little effect on the immature cotton rat, while exposure to constant darkness causes a definite retardation in the development of the reproductive tract and maturation. The latter observation is similar to that of Fiske (1941), who found that immature female white rats kept in darkness came into sexual maturity, as evidenced by vaginal introitus and the weight of the reproductive organs, later than did rats kept under normal laboratory conditions. However, her immature female white rats kept in continuous light matured somewhat earlier than did the controls. Perhaps light duration and intensity, once certain values have been reached, are of no further significance to the immature cotton rat. If this is true, the light available to the control animals in the vivarium must have been above this minimal value in duration and intensity.

The most widely favored theory offered as an explanation of the effect of light on the reproductive activity of animals has been that the eye, acting as a light receptor, stimulates the pituitary, presumably by a nervous pathway, which in turn induces gonadal development (Llewellyn, 1932; Marshall and Bowden, 1934; Benoit, 1934; and Bissonnette, 1936b). Fiske presents evidence to indicate that light stimulates the pituitary to increase the production and release of FSH, while a lack of light causes a decrease in FSH and, in the female only, an increase in LH secretion. Thus, according to this

hypothesis, the abnormally small amount of FSH secreted by the pituitaries of the rats raised in darkness would account for the retarded development of the reproductive tracts of these animals.

EFFECT OF CONSTANT LIGHT OR CONSTANT DARKNESS ON THE VAGINAL ESTROUS CYCLE OF THE ADULT COTTON RAT

A total of 9 adult female cotton rats were used for this study. These animals were among those which had been used to establish the normal vaginal estrous cycle (Meyer and Meyer, 1944) so that the cycle of each of these individuals under ordinary laboratory conditions was known. All of the animals were over 100 days of age when this study was started. Five of the rats were placed in constant light and 4 in constant darkness for a period of 72 days. Vaginal smears were taken daily from the animals of both groups over the last 58 days. At the end of this period the groups were interchanged so that the rats previously in darkness were kept in light and those previously in light were kept in darkness, for a period of 45 days. Vaginal smears were taken daily from the animals of both groups over the last 31 days. The term "constant darkness' is used with reservation as the animals kept in the dark were exposed to light for several minutes once daily at the time when vaginal smears were taken.

Our results show no significant difference between the average length of vaginal estrus or vaginal diestrus of the animals kept in light and of those kept in darkness. While Fiske found that the estrous cycles of white rats kept in light exhibited unusually long periods of estrus and those of animals kept in the dark showed longer periods of metestrus than did those of normal rats, her work was done with animals which were not fully mature. It may be that the pituitary of the adult cotton rat is less susceptible to variation in light than that of the less mature animal, and it would seem worth while to repeat this experiment using young females kept under the experimental conditions from the time of, or before, vaginal introitus.

The average lengths of the estrous cycles of the animals in both experimental groups were shorter, in 8 of 9 cases, than those taken from the same rats when they were housed in the vivarium. This difference was due, for the most part, to the longer periods of vaginal estrus shown by the animals while they were in the vivarium. While in the vivarium, the average period of vaginal estrus shown by these rats was 3.2 days, the average period of diestrus (including pro- and metestrus) was 5.9 days and the average length of the cycle was 9.1 days. During this study, the average period of vaginal estrus shown by these rats was 1.6 days, the average period of vaginal diestrus was 5.2 days and the average length of the cycle was 6.8 days. Since there was much less disturbance in the room used for this study than in the vivarium, these observations seem to offer additional evidence that

environmental disturbance has a definite effect on the estrous cycle of the cotton rat (Meyer and Meyer, 1944).

SUMMARY

Exposure to constant light from birth, under the experimental conditions, had little effect on the immature cotton rat, while exposure to constant darkness had a definitely retarding effect on the development of the reproductive tract and maturation.

There was no significant difference between the average lengths of vaginal estrus or vaginal diestrus of adult female cotton rats kept in light and of those kept in darkness.

Additional evidence is presented to show that environmental disturbance has an effect on the estrous cycle of the cotton rat.

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EFFECT OF THIOURACIL ON THE IODINE CONTENT OF THE THYROID GLAND[1]

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IT IS NOW well established that anti-thyroid drugs such as thiouracil prevent the formation of thyroid hormone. As the mechanism of this action is still obscure it seemed likely that studies on the iodine metabolism of the thyroid gland would provide useful information. In view of the fact that the iodine content of the thyroid gland reflects the quantity of active principle contained therein, measurements of the iodine concentration under various experimental conditions might be expected to define more exactly the changes brought about by anti-thyroid drugs in the synthesis of thyroid hormone.

Abnormally low concentrations of iodine in the thyroid have been noted in the simple colloid goiters which occur in regions of iodine deficiency, in the hyperplastic glands of hyperthyroid patients, in the goiters experimentally produced by iodine deficient and goitrigenic diets, and in the hyperplastic thyroids resulting from the injection of pituitary extracts. Thus with the exception of certain colloid goiters, low iodine values are associated with a state of hyperplasia and a decrease in the colloid in the gland. Thiouracil treatment not only inhibits the formation of thyroid hormone but in certain animals such as the rat this is followed by a marked compensatory hyperplasia. These two factors combine to give the marked changes in iodine contration noted below.

METHODS

Young male and female rats averaging 100 grams in weight were given thiouracil[2] by dissolving it in the drinking water. The maximal concentration used was 0.1%, an amount which would remain in solution for several days at room temperature without the addition of alkali after being dissolved in warm distilled water. In experiments involving time relationships this treatment was started by a single subcutaneous injection of an aqueous suspension of the thiouracil powder. The fine powder was added to a warm 0.1%

Received for publication February 9, 1944.

[1] This work was carried out under the auspices of the University Committee on Pharmacotherapy and was aided in part by a grant from the Committee on Endocrinology of the National Research Council.

[2] Supplied by Dr. B. W. Carey of the Lederle Laboratories, Inc., Pearl River, New York.

agar solution and the whole chilled in ice while shaking vigorously. The final suspension contained 100 mg. per cc.; the solid phase settled slowly but could be uniformly resuspended by shaking. One quarter of a cc. of this suspension provided a depot of thiouracil which was slowly absorbed over several days.

Iodine determinations were made on the individual, freshly dissected and weighed thyroid glands by the method of Kendall (1914, 1920) slightly modified for the micro quantities of tissue available. Ashing was done with potassium hydroxide in nickel crucibles; the solution was filtered, acidified, oxidized with bromine and titrated after the addition of potassium iodide with 0.001 N sodium thiosulfate solution in an ice bath to a starch end point. Frequent blank determinations on the redistilled water and reagents consistently showed no detectable iodine. The method was accurate to about 0.2 μg. of iodine; amounts smaller than this could not be detected. Repeated checks on the iodine content of standard solutions of organic iodine compounds such as thyroxin showed that the method had an error not greater than 0.2 μg.

The animals were fed on Purina fox chow which was found to contain 1.88 μg. of iodine per gram. This concentration of iodine assured a daily dietary intake of roughly 20 μg. of iodine per 100 gm. of body weight, or many times the minimal daily requirement for this species (Levine, Remington and von Kolnitz 1933, Cole and Curtis 1936). In all experiments the animals were kept in a room the temperature of which was maintained near 25° C.

RESULTS

Time relationships. Several groups of rats received 25 mg. of thiouracil in a single subcutaneous injection of the suspension and were then given a 0.1% solution of the drug as drinking water. They were sacrified at 24 hour intervals, and their thyroids were removed for iodine determinations. The individual results were plotted (fig. 1). At the end of the first 24 hours there was a definite decrease of iodine, and for 5 days this decrease of iodine, both relative and absolute, continued in a regular manner. At the end of this time the iodine concentration had fallen to one-thirtieth the normal value, a level which was maintained for as long as the drug was given (five months).[3] In those instances where the drug was given for 8 days only and then stopped, it was found that iodine quickly returned to the thyroid: there was a significant increase in 24 hours; but, though the gain continued, the concentration was still less than normal at the end of a week and individual variations were considerable.

The weight of the thyroid, under the same conditions, changed more slowly: it increased slightly in the second 24 hours, but this was not statistically significant. At the end of 72 hours there was a definite

[3] After 5 days of treatment the iodine content of a single gland approaches the limit of the iodine method. A more accurate estimate of the level to which the iodine concentration falls was provided by estimations on the iodine content of the pooled glands of 3 groups of rats which had been treated for 10 days with thiouracil in the form of a 0.1% solution in the drinking water. The total iodine averaged 0.31 μg. per gland and the concentration 1.43 mg. per 100 gm. of fresh tissue.

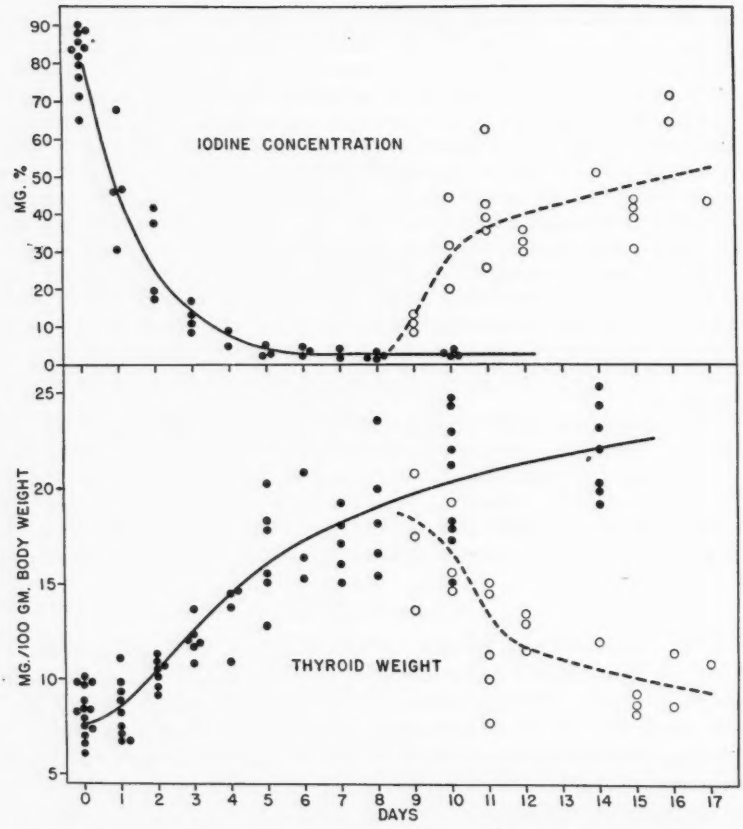

FIG. 1. Changes in iodine concentration of the thyroid gland (upper curves) expressed in mg. per 100 gm. fresh tissue, and thyroid weight (lower curves) expressed in mg. per 100 gm. of body weight. Thiouracil was administered in the form of a 0.1% solution in the drinking water following an initial subcutaneous injection of 25 mg. of a thiouracil suspension. Each point represents the value of a single determination on one animal. The solid lines and circles show the effects of continued treatment while the dashed lines and open circles represent the effects of withdrawing the drug after eight days.

increase in weight (+50%); weight gain continued rapidly for the next 5 days and then more and more slowly. When the drug was stopped after 8 days, the thyroids lost weight quickly and reached normal size within 7 days. This is in contrast to the subnormal values for iodine.

Because of variation in the size of the animals and in the weights of the thyroid glands, the total iodine content was found to be more variable within a group of animals than the iodine concentration. Figure 1 shows the decrease in the relative iodine content of the

enlarging thyroid gland. It is obvious from this plot that in terms of total iodine content in the whole thyroid gland the changes are similar in direction and time relationship but less in magnitude.

Prolonged treatment. When thiouracil was given for longer periods than those shown in figure 1 little further increase in the thyroid weight-body weight ratio was noted and no further decrease in iodine concentration was found.

Six male rats of the same litter, 25 days old, were treated as shown in table 1. Two received 0.01% thiouracil in their food; two received 0.1%; and two were untreated. At the end of 9½ months those given 0.01%, far from being smaller, were actually heavier than the controls, although their body and tail lengths were normal. Their thyroids were only about twice the normal size, but the iodine concentration was reduced to a tenth. The rats on the larger dose were considerably smaller than the controls; their thyroids were about six times the normal size and were nearly free of iodine.

TABLE 1. EFFECT OF 9½ MONTHS OF THIOURACIL TREATMENT FROM THE 25TH DAY OF AGE ON THE WEIGHT AND IODINE CONTENT OF THE THYROID GLAND OF MALE RATS

Concentration of Thiouracil in Food	Body Wt.	Thyroid Wt.	Thyroid Wt.	Total Iodine	Iodine Conc.
%	*gm.*	*mg.*	*mg./100 gm. body wt.*	*μg.*	*mg./100 gm. wet tissue*
0	356	29.8	8.4	33.4	112.0
0	364	29.0	8.0	35.7	123.0
0.01	437	69.6	16.0	7.7	11.1
0.01	417	70.6	17.0	12.1	17.1
0.10	226	129.0	57.0	1.5	1.2
0.10	257	113.4	44.0	1.8	1.6

Effect of thyroxin and of hypophysectomy. In table 2 are shown the influences of thyroxin treatment and of hypophysectomy on these changes. It may be noted that a daily dose of 10 μg. of thyroxin for 1, 5 and 10 days in animals which had previously received thiouracil for 30 days and which continued to receive it during the thyroxin treatment, caused a reduction in the size of the thyroid but did not permit a reaccumulation of iodine. Hypophysectomy under similar conditions caused a marked and rapid reduction in the weight of the thyroid but did not alter the low iodine content of these glands. Similar results were obtained whether of not the thiouracil was continued or discontinued after hypophysectomy.

Thus it would appear that ablation of the hypophysis or the suppression of its thyrotropic function by thyroxin injections greatly hinders the thyroid gland's ability to reaccumulate iodine after it has been depleted. Similarly it may be seen that if hypophysectomy were performed before thiouracil was given no loss of iodine occurred in a

period of 7 days of treatment. These observations are in accord with the findings of Morton, Pearlman, Anderson and Chaikoff (1942) who have shown that hypophysectomy greatly retards the uptake of radioactive iodine by the thyroid gland and impedes the synthesis in the gland of diiodotyrosine and thyroxin.

The concurrent administration of thyroxin to thiouracil-treated rats completely prevents the hyperplasia that normally follows the administration of an anti-thyroid drug (Mackenzie and Mackenzie 1943, Astwood, Sullivan, Bissell and Tyslowitz 1943). The dosage of thyroxin necessary to maintain the thyroid gland at a normal size

TABLE 2. SOME EFFECTS OF HYPOPHYSECTOMY AND THYROXIN TREATMENT ON THE WEIGHT AND IODINE CONTENT OF THE THYROID GLANDS OF THIOURACIL-TREATED RATS

Thiouracil Pre-treatment	Other Treatment	Thyroid Wt.	Total Iodine	Iodine Conc.
		mg./100 gm. body wt.	*μg.*	*mg./100 gm. wet tissue*
30 days (5 rats)*	None	28.9	0.5	1.3
30 days	10 μg. Thyroxin 1 day Thiouracil continued	26.6	0.4	1.4
30 days	10 μg. Thyroxin 5 days Thiouracil continued	19.9	0.6	2.3
30 days	10 μg. Thyroxin 10 days Thiouracil continued	21.7	0.5	1.9
5 months	None	82.7	1.8	1.4
5 months	Hypophysectomized 7 days Thiouracil continued	47.1	0.9	2.2
5 months	Hypophysectomized 14 days Thiouracil continued	22.0	1.6	7.3
None	Hypophysectomized 7 days	11.3	14.0	130.0
None	Hypophysectomized and Thiouracil 7 days	8.4	11.7	135.0
8 days (3 rats)	No treatment 7 days	8.7	5.9	38.1
8 days	Hypophysectomized and Thiouracil 7 days	12.2	0.5	4.9
	Hypophysectomized and Thiouracil 7 days	10.5	2.8	28.4
8 days	Hypophysectomized 7 days Thiouracil omitted	8.8	2.4	25.2
	Hypophysectomized 7 days Thiouracil omitted	8.5	1.1	11.9

* The values are for single animals except when otherwise stated.

under thiouracil treatment is so precisely balanced that this type of experiment has been used to estimate the normal thyroid hormone requirements and can serve as an assay method for thyroid hormone (Dempsey and Astwood 1943).

Measurements of the iodine of the thyroid glands of rats treated for 10 days with thiouracil and also with various dosages of thyroxin are shown in table 3 in which it may be seen that a daily dose of 2.5 μg. for ten days slightly impeded the hypertrophy of the gland but did not modify the loss of iodine. A dose of 5.0 μg. daily maintained the gland at a normal size but only moderately inhibited its loss of iodine

while with a dose of 20.0 μg. daily the thyroid atrophied and there was little if any loss in iodine.

Effect of injections of thyrotropin. It has been clearly demonstrated that the injection of pituitary extracts containing thyrotropin results in a decrease in the iodine concentration in the thyroid gland (Closs, Loeb and MacKay 1932; Schockaert and Foster 1932, Foster 1934), and it appears not unreasonable to assume that at least a part of the loss of iodine following thiouracil administration is attributable to the compensatorily-induced excessive thyrotropin stimulation. An attempt was therefore made to induce a loss of iodine of a comparable degree by thyrotropin injections alone and to determine whether this would be influenced by concurrent thiouracil administration.

TABLE 3. INFLUENCE OF THYROXIN ON THE LOSS OF IODINE FROM THE THYROID GLANDS OF THIOURACIL-TREATED RATS*

Daily Dose of Thyroxin	Thyroid Wt.	Iodine Conc.
μg.	*mg./100 gm. body wt.*	*mg./100 gm. wet tissue*
0	16.9	4.3
2.5	13.8	4.8
5.0	7.7	23.6
20.0	4.6	75.1
Controls	7.4	80.2

* Each value represents the average of 3 animals.

A purified thyrotropin preparation[4] was administered subcutaneously in a dose of 10 mg. twice daily to normal young male rats and to like animals which also received thiouracil as a 0.1% solution in the drinking water. This dosage of thyrotropin was equivalent to about 60 guinea pig units daily and when such dosages of the preparation were used, pronounced gonadotropic effects were apparent. The rate of increase in size of the thyroid glands (table 4) exceeded considerably that which followed thiouracil administration (cf. fig. 1). Three days of treatment doubled the weight of the thyroid gland, and the concurrent administration of thiouracil did not cause a further increase in weight. It can probably be assumed that this dose of thyrotropin induced a maximal rate of growth in the thyroid gland. The rate of decrease in thyroid iodine concentration induced by the thyrotropin was considerably slower than with thiouracil alone and the two substances given simultaneously caused the iodine concentration to fall at a rate which was no faster than that of thiouracil by itself. Because of the marked changes in thyroid size in this experiment, the alterations in the total iodine of the thyroid gland are of interest. The glands of animals receiving thyrotropin alone contained nearly as much iodine as the controls while those which received both thiouracil and

[4] A powdered preparation containing 3–4 Junkmann-Schoeller units per mg. supplied by Dr. D. A. McGinty, Parke, Davis & Co., Detroit, Michigan.

thyrotropin lost about one half of their iodine content, much less than did animals receiving thiouracil alone. It would appear from this experiment that thyrotropic hormone may, under certain conditions, aid in the deposition of iodine in the gland.

It has been well established by the use of radioactive iodine that the injection of thyrotropic hormone increases the rate at which iodine is taken up by the thyroid gland (Hamilton and Soley 1940; Hertz, Roberts, Means and Evans, 1940; Leblond and Sue 1941; Morton, Perlman and Chaikoff, 1941). This action of thyrotropin might be considered to be opposed to the effects of thiouracil upon the forma-

TABLE 4. EFFECT OF LARGE DOSES OF THYROTROPIN ON THE IODINE CONTENT OF THE THYROID GLAND

	Days Treated	No. of Rats	Thyroid Wt.	Iodine Conc.	Total I_2 per Gland
			mg./100 gm. body wt.	*mg./100 gm. wet tissue*	*μg.*
Controls		4	9.3	77.4	5.0
10 mg. Thyrotropin twice daily	1	1	8.5	44.7	2.9
	2	2	14.0	38.7	4.3
	3	2	18.0	55.9	8.0
	4	2	22.9	28.7	3.1
10 mg. Thyrotropin twice daily 0.1% Thiouracil in drinking water	1	1	8.4	37.6	2.9
	2	2	12.9	16.3	1.4
	3	2	16.8	21.5	2.8
	4	2	19.1	14.5	2.4

tion of thyroid hormone. Thiouracil would thus be less effective in inhibiting thyroid hormone synthesis in the presence of excessive quantities of thyrotropin. It should be pointed out, however, that some of the observed changes following the injection of large amounts of thyrotropin may be due to other substances contained in the extract or to non-specific toxic effects of the preparation employed. Though a large decrease in iodine concentration of the thyroid gland can be induced by the injection of thyrotropic hormone in normal animals this decrease is much less than that following the administration of thiouracil. From the values shown in table 4 it would appear that only a part of the loss of thyroid iodine consequent to thiouracil administration can be attributed to an excessive thyrotropic stimulus.

Factors influencing the reaccumulation of thyroid iodine. Foster (1934) has shown that the thyroid gland of the guinea pig, when depleted of its iodine by treatment with thyrotropic hormone, reaccumulates iodine at a very slow rate unless extra iodine is supplied either in the form of potassium iodide or as diiodotyrosine. Under the conditions of the experiment recorded in figure 1 recovery of iodine was much more rapid. Two possible explanations for this suggested

themselves. In the first place the diet used contained an amount of iodine considerably in excess of the normal requirement and it is possible that a normal diet for a guinea pig such as the one used by Foster contained less iodine. A second possible explanation was suggested by the apparent differences in the degrees of thyrotropin activity in the two species. The thyroid gland of the guinea pig is recognized to be much more sensitive than that of the rat to exogenous thyrotropin and yet the guinea pig gland appears histologically to be much less active than that of the rat. These considerations suggest that the guinea pig hypophysis secretes relatively very little thyrotropin while the rat hypophysis secretes thyrotropin in relatively large amounts.

The effect of dietary iodine intake on the reaccumulation of iodine in the thyroid gland was studied on three groups of three rats each of which had received thiouracil for 8 days. The thiouracil was discontinued for a period of 4 days in all three groups and during this time one group was given the stock diet, one, this diet plus added iodine in the form of a 0.1% solution of potassium iodide in the drinking water and the third group was totally starved. The average iodine concentrations at the end of the 4 days in terms of mg. per 100 gm. fresh thyroid tissue were 37.8 and 38.2 respectively for the first two groups indicating that the added iodine had no effect on the reaccumulation of iodine while in the starved group the value was 19.3, representing a distinctly smaller reaccumulation.

Comparative effect of Sulfadiazine. Substances which inhibit the formation of thyroid hormone have been shown to fall into two general classes: those related to thiourea of which thiouracil is an example and those possessing an aniline group in addition to some other, as yet poorly defined, component (Astwood, 1943). The most active compound of this second group has thus far proven to be sulfadiazine which is nearly as effective as thiourea. These two groups of agents differ so greatly in structure as to suggest that their site and mechanism of action in inhibiting thyroid hormone formation may be different. For this reason the effect of large doses of sulfadiazine upon the rate of loss of iodine from the thyroid gland was studied.

Two groups of male rats of equal age and average body weight were treated simultaneously. One received thiouracil in the form of a 0.1% solution in the drinking water and the other sulfadiazine admixed with the food in a concentration of 0.5%. These dosages were roughly comparable as far as could be judged from previous experiments on size and structural changes in the thyroid gland. Determinations of the weight and iodine content of the thyroid glands were made on animals killed at intervals for 8 days, and on animals subsequently treated for 8 days with 10 μg. of thyroxin daily. The values observed are shown in table 5 from which it appears that there was little difference in either the rate of growth of the thyroid gland or in

the rate of loss of iodine. The small differences in the two groups can hardly be cited as evidence of any real qualitative difference in this action of the two classes of drugs. The rate of recovery when thyroxin was administered was more rapid in the case of sulfadiazine in this experiment.

It is of interest to compare the rate of the changes in weight and iodine concentration shown in figure 1 with those of table 5. In the former case an injection of a thiouracil suspension initiated the treatment while in the latter experiment this injection was omitted. The rate of change in the latter group of animals was slower than that of the former by a period of about 24 hours, a phenomenon that is probably to be explained by a delay in attaining an effective concentration of the drug in the body tissues after oral administration.

TABLE 5. COMPARISON OF THIOURACIL AND SULFADIAZINE IN THE RATE OF LOSS OF IODINE FROM THE THYROID GLAND

Days Treated	0.5% Sulfadiazine in Food			0.1% Thiouracil in Water		
	No. of Rats	Thyroid Wt.	Iodine Conc.	No. of Rats	Thyroid Wt.	Iodine Conc.
		mg./100 gm. body wt.	*mg./100 gm. wet tissue*		*mg./100 gm. body wt.*	*mg./100 gm. wet tissue*
0	3	6.7	84.9	3	6.7	84.9
1	2	6.3	56.9	2	7.0	68.0
2	2	6.9	40.3	2	7.3	43.8
3	2	8.9	21.8	2	8.8	38.3
4	2	9.5	25.0	2	12.8	15.8
8	2	15.3	4.8	2	16.1	4.3
16	1	18.0	5.3			
16	2*	6.2	30.5	2	7.4	7.0

* Animals given 10μg. thyroxin daily during the last 8 days of the experiment.

Partition of the thyroid iodine. It is generally accepted that the major portion of the iodine in the normal thyroid gland is in the form of an organic complex presumably of a protein nature. As the loss of iodine after thiouracil treatment is so nearly complete it was of interest to determine what fraction of the remaining iodine and what proportion of the lost iodine behaved as protein bound or organic iodine. Thyroid glands from normal animals and from animals treated for 2 weeks were ground in a glass homogenizer and suspended in a 1% sodium chloride solution. After stirring for one half hour one third of each suspension was removed for determinations of the total iodine, and the remainder was precipitated with Somogyi's reagents (Somogyi, 1930). Iodine determinations were made on the thoroughly washed precipitates and on the supernatants and washings. The values found are summarized in table 6. A small loss was incurred in the separate determinations and only 94% and 87% of the total iodine was recovered when the two fractions were added together. However it was apparent that the major portion of the iodine was contained in the washed precipitates. Calculated on the basis of the actual deter-

mination on the precipitates and supernatants 98.7% of the iodine of normal glands and 93.4% of the iodine of the treated glands was in a form which precipitated with the protein fraction. Although the amount of iodine in the treated glands was so small that a considerable error was involved in the measurements, it appears that the depleted glands contained a considerably larger proportion of readily soluble iodine than the normal glands. This finding would agree with the assumption that a very small proportion of the total iodine in the normal gland is in a form which behaves as though it were inorganic. This fraction may be presumed to be unrelated to the store of thyroid hormone and not influenced by thiouracil treatment. Were this fraction to make up an estimated 1% of the total iodine of the normal gland, it would account for the larger proportion of inorganic iodine remaining in the depleted thyroid glands of thiouracil-treated animals.

TABLE 6. DISTRIBUTION OF IODINE BETWEEN "ORGANIC" AND "INORGANIC" FRACTIONS IN THE THYROID GLANDS OF NORMAL AND THIOURACIL-TREATED RATS

	No. of Rats	Average Thyroid Wt.	Total Iodine per Gland	Organic Iodine per Gland	Inorganic Iodine per Gland
		mg.	*μg.*	*μg.*	*μg.*
Normal	6	9.33	8.46	7.83	0.11
Treated 2 weeks	6	27.62	0.85	0.69	0.05

Effect of dosage. Although slight degrees of thyroid hyperplasia are detectable histologically such changes are difficult to quantitate. In previous experiments on the relative effectiveness of a number of antithyroid compounds, the histological method was used and it was apparent that a more objective and more sensitive method was desirable. Mackenzie and Mackenzie (1943) have shown that the increase in thyroid weight in two weeks is directly proportional to the concentration of sulfaguanidine in the diet over a considerable range of dosage. A method for expressing the activity of a drug based upon objective criteria such as weight or iodine concentration would be more accurate and satisfactory than one based upon histological changes.

Two sets of experiments have been done on the effect of decreasing doses of thiouracil on the iodine content of the thyroid gland. The drug was dissolved in the drinking water at concentrations of from 0.1% to 0.0001%, and determinations of the resulting weight and iodine concentration of the thyroid were carried out at the end of 5 and 10 days. In these experiments no initial subscutaneous dose was given. From figure 2 it may be seen that, whereas concentrations of 0.03 to 0.1% for 5 days were required to double the thyroid weight, a concentration of 0.003% caused a marked decrease in the concentration of iodine.

The sensitivity of this reaction was greatly increased by prolonging the period of treatment to 10 days, and here again the iodine con-

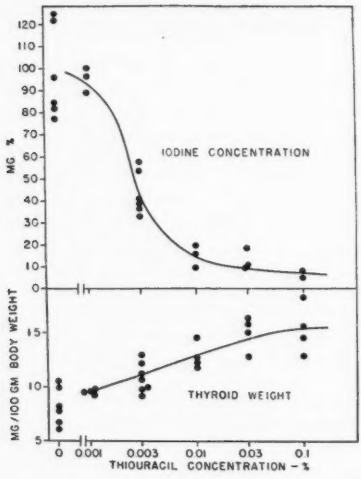

FIG. 2. Effects of dosage of thiouracil on the iodine concentration and weight of the thyroid gland after five days of treatment. Thiouracil was administered in the drinking water at the concentrations shown in the abscissa (logarithmic scale). Each point represents a single animal.

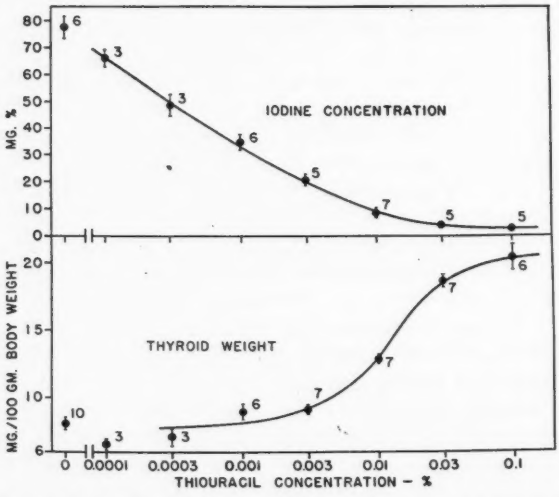

FIG. 3. Relation of dose of thiouracil to the iodine concentration and the weight of the thyroid gland after ten days of treatment. Thiouracil was administered in the drinking water in the concentrations shown in the abscissa (logarithmic scale). The figures beside the points represent the number of determinations and the vertical lines above and below each point are the standard errors of the means. The curves were fitted to the data by eye.

centration was a much more delicate indicator than increase in weight (fig. 3). A significant weight increase required a concentration of from 0.003% to 0.01% while a marked decrease in iodine followed the administration of 0.0003%. Thus the loss of iodine is brought about by less than one tenth the quantity of thiouracil required for a weight increase or a definite histological change in the thyroid gland. The data from which figure 3 was constructed were collected at intervals over several months, and the values obtained were found to be reproducible within narrow limits as indicated by the size of the standard errors shown in the figure. These two curves have served as standards for the determination of the relative anti-thyroid activity of new compounds. The five day experiment shown in figure 2 was found to be less satisfactory for this purpose.

The weight of the thyroid glands after 10 days of treatment with concentrations of 0.001% and 0.0003% were found to be lower than the normal control animals in this experiment. The difference though small is, in the case of the smaller dose, statistically significant. However, from these few data it is not certain that this is a general phenomenon.

DISCUSSION

The rapid loss in iodine from the thyroid gland following the initiation of thiouracil treatment appears, from the data in figure 1, to be a regular and even process with a constant proportion of the contained iodine lost each day. Assuming that thyroid hormone synthesis is completely inhibited at the time treatment is started, one would expect that the rate of loss of iodine at that moment would be related to the normal rate of secretion of the thyroid hormone. To test this hypothesis the averages of the data for the first few days of treatment were plotted as in figure 4: time against the log concentration of iodine. A straight line was fitted to the data of the first five days by the method of least squares. The slope of this line at the start of treatment, obtained by taking the first derirvative of the equation and substituting zero time, defines the rate of change in iodine concentration at the start. This calculation shows that the initial rate of iodine loss is such that 50.8% of the contained iodine would be lost in the first 24 hours. Assigning an average thyroid weight of 7.4 mg. with an iodine concentration of 81.25 mg.% for a normal rat weighing 100 gm. the actual loss of iodine amounts to 50.8% of 6.01 μg. or approximately 3μg. per day. Were this iodine in a form similar to thyroxin the daily thyroid hormone production would be equivalent to 4.8 μg. of thyroxin. This figure is in agreement with a previous estimate (5.2 μg.) of the daily thyroid hormone requirement of animals of this size maintained under similar environmental conditions (Dempsey and Astwood). Inasmuch as these two determinations made by such different methods are in close agreement, support is lent to the hypothe-

sis that thyroid hormone production ceases promptly and nearly completely when thiouracil is given.

The exact site of action of thiouracil in inhibiting thyroid hormone synthesis is not established by these experiments. It is clear, however, that evidence of thyroid inhibition extends back to the process of accumulation of iodine in the gland. It seems not unlikely that most of the iodine in a normal gland is in the form of thyroxin, a component of the thyroglobulin molecule, and it is possible that a break at any point in the chain of events leading to thyroid hormone production would

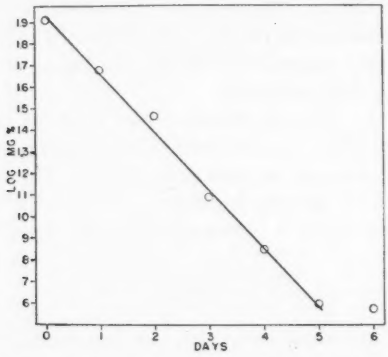

FIG. 4. Changes in the iodine concentration of the thyroid gland plotted logarithmically against time following the initiation of thiouracil treatment (data from fig. 1). The curve was fitted to the data of the first five days by the method of least squares, yielding the expression: $y = 1.91 - 0.25x$ where y is log. concentration of iodine and x the duration of treatment in days.

inhibit the other reactions by the princple of mass action. Consequently these data cannot be considered proof that the primary site of action is concerned with the process of iodination of tyrosine residues or with the selective fixation of iodine in the thyroid gland. Similarly, although the two main classes of anti-thyroid compounds—thiourea and aniline derivatives—might be expected to differ in their mechanism of action in inhibiting the formation of thyroid hormone, no qualitative difference on the loss of thyroid iodine could be detected by the methods used.

The observed changes in iodine concentration in the glands of thiouracil treated rats confirm the observations of Gersh and Baker (1943) who have shown that treatment with sulfaguanidine decreases both the total protein and the organic iodine concentrations in the colloid of the thyroid gland. By absorption spectrophotometry on histological sections these workers also observed a great increase in the organic iodine of hypophysectomized rats treated with sulfaguanidine. The data on animals treated with thiouracil shown in table 2

indicate that this compound does not modify the iodine concentration after hypophysectomy.

The decrease in iodine concentration following the administration of thiouracil to normal rats follows closely the loss of colloid from the glands. This is what one would expect if the colloid material contained the major part of the stored thyroglobulin. This concept is not applicable, however, to certain other observations recorded above. Van Dyke (1921, 1922), using a method similar to that developed by Tatum (1920), showed clearly that the major portion of the iodine contained in thyroid glands in various states of activity could be readily dissolved by saline solutions in which thin sections of the gland were suspended, providing strong evidence that the major store of thyroid hormone in the gland is contained in the colloid. This treatment resulted in the separation from the cellular elements of the gland the readily soluble material including the colloid and the intercellular fluid. The possibility was not excluded that iodine containing materials were also leached out of the follicular cells—a possibility which would allow a more ready explanation of some of the data reported above.

The administration of thyrotropic hormone, while inducing a maximal degree of hyperplasia and a nearly complete loss of stainable colloid material from the glands, did not reduce their iodine content to a degree comparable to that which followed thiouracil administration. The remaining iodine in the glands of thyrotropin treated animals may have been contained in the enlarged follicular cells. This view is in accord with the observations of Bensley (1916) that the follicular cells contain droplets of colloid-like material reported by Gersh and Caspersson (1940) to give an absorption spectrum similar to thyroglobulin. Similar droplets have been described by Williams (1937, 1939) in living thyroid cells and their appearance was found to be accelerated by thyrotropin injections.

A more striking discrepancy with the concept that the quantity of stainable colloid parallels the hormone content of the gland is to be found in the experiments on factors which modify the reacculmulation of iodine in thyroid glands depleted by thiouracil treatment. When thiouracil had been given for a period of eight days and the iodine concentration reduced to a minimum, the administration of thyroxin or the removal of the pituitary was followed by a reaccumulation of densely-staining colloid material. This protein, which is presumably newly-formed, must have been virtually free of thyroid hormone as the quantity of iodine in the whole gland remained low. Dempsey (1944) has shown that colloid formed under these conditions exhibits abnormal fluorescence when viewed under ultraviolet light, a further evidence of its low hormone content.

SUMMARY

The administration of thiouracil to young rats was followed by a nearly complete disappearance of iodine from the thyroid glands in five days and a threefold increase in the size of the glands in two weeks. These effects were inhibited by hypophysectomy or by the injection of thyroxin. Withdrawal of the drug after eight days of treatment was followed by a prompt decrease in the size of the glands and a rapid reaccumulation of iodine. Injections of thyroxin or removal of the pituitary markedly retarded the accumulation of iodine; the glands decreased in size, became hypoplastic and filled with colloid. The colloid formed under these conditions must have been nearly free of thyroid hormone as such glands contained very small amounts of iodine. Similar effects followed the administration of sulfadiazine.

The injection of large amounts of thyrotropic hormone induced a marked enlargement of the thyroid glands of normal rats but caused a relatively small loss of iodine. Thyrotropin delayed slightly the loss of iodine from the thyroid glands of rats treated concurrently with thiouracil.

Graded doses of thiouracil administered for ten days resulted in proportional changes in the weight and iodine concentration of the thyroid glands. The relative anti-thyroid activity of new compounds can be determined in this way.

These data support the view that thiouracil inhibits the formation of thyroid hormone but do not establish the mechanism of this effect.

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NOTES AND COMMENTS

YOLK ESTROGEN AND HUMERUS PNEUMATIZATION IN CHICKS

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IT IS WELL known that in fowl the humerus contains an air sac. This pneumatization of the humerus does not exist in newly-hatched chicks and the changes coincident to ingrowth of the air sac are not initiated until between 12 and 20 days after hatching.

It has been suggested by Bremer (1940) that "the estrogens taken into the chick by the retraction of the yolk sac before hatching act either directly or indirectly on the parathyroid glands to produce a temporary condition of osteitis fibrosa, thus affording a mesenchymal pathway for the air sac with resulting pneumatization of the bones." This hypothesis was based: (1) on the similarity existing between the histological processes which characterize ingrowth of the air sac into the humerus and those seen in the long bones of mammals after treatment with estrogen; and (2) on the presence of estrogen in the unresorbed yolk of chicks.

It is a fact that the yolk of chicks contains appreciable amounts of estrogen. It is undeniable also that the histological changes which precede and accompany pneumatization bear a fair degree of resemblance to the pathological phenomena called forth in avian and mammalian bone by estrogen administration.

Yet these two isolated facts can hardly be said to constitute even a modicum of "evidence" for a causal relationship between the presence of estrogen in the yolk and the pneumatization of the humerus. Indeed, there is evidence to the contrary. Bremer himself was unable to prevent or delay the occurrence of pneumatization by injection of androgen. Again, it is well established that bone abnormalities, brought forth by estrogen administration, are promptly reversed when treatment with the hormone is discontinued. The structure of the bone returns rapidly to normal. Yet, pneumatization persists in

Received for publication December 21, 1943.

the humerus after resorption of all yolk—even in capons and poulards. The assumption that the small amount of estrogen which the chick can obtain from the yolk produces *lasting* histogenetic effects would need rigorous experimental support before it could be accepted.

The obvious experiment in this situation would seem to be removal of the yolk sac from chicks. Against this Bremer advanced the following argument: "What might be called the direct method of testing this theory, by removal of the yolk sac from the shell before it was retracted, did not seem appropriate in this case, because any change from normal which might be obtained could be perhaps interpreted as merely the result of malnutrition due to the loss of so much normal food." While it is true that failure of pneumatization to occur in such a situation would not constitute conclusive evidence for the assumed rôle of estrogen, undisturbed pneumatization of the humerus after yolk removal surely is definite evidence against the assumption. Moreover, while the removal of the yolk might be attended by difficulties before hatching, it can readily be done as soon as the chicks are hatched. Such an operation is not a cause of serious handicaps to chicks (Parker, 1929; unpublished observations).

In earlier experiments (Landauer, 1937), involving 38 male chicks, the yolk sac and the testes were removed at hatching time. The chicks were then raised to the age of ten months. They were sacrificed at that time for observations on the long bones. Pneumatization of the humerus existed as in normal controls.

New experiments have now been made in which, on the day of hatching, the yolk sac was removed from six newly hatched White Leghorn chicks. Six other chicks were kept as controls. Three chicks of each group were killed at the age of 21 and 25 days, respectively. The average body weights of these chicks are shown in the following table.

Group	At Hatching (prior to removal of yolk) N=6	At Age 21 Days N=6	At Age 25 Days N=3
	g.	*g.*	*g.*
Yolk sac removed	38.8	108.0	115.7
Control	40.1	117.8	126.7

Sections were made of one humerus of each chick from which the yolk sac had been removed and of four of the control chicks. Pneumatization was present in the humerus of all chicks, without exception. There was no consistent difference between control and operated chicks in the degree of pneumatization which had been attained. In addition, we had one chick in which both the yolk sac and the testes had been removed at hatching time. It was killed when 21 days

old. Sections showed that pneumatization of the humerus was under way.

It is known from experimental work that estrogen produces its effects on bone within a very short time after being introduced into the circulation. Hence, it cannot be said that, prior to removal of the yolk sac, sufficient yolk estrogen had already been resorbed to induce pneumatization. We conclude that pneumatization of the humerus of chicks is not under the control of yolk estrogen.

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BOOK REVIEW

PHYSIOLOGICAL PSYCHOLOGY. By Clifford T. Morgan, Associate in Psychology, The Johns Hopkins University. McGraw-Hill Book Co., New York. $4.00. First Edition, 1943, xii+623 pp., 176 figures. Cloth.

This is "meant to be both a textbook for undergraduates who are preparing for psychology or medicine and a reference book for graduate students and workers in psychology, physiology, and medicine."

The first one hundred pages contain an introductory chapter on the history of physiological psychology and four additional chapters on cellular functions, the internal environment of cells provided by the blood, the physiology of nerve cells, and the structure and functions of the nervous system. Then follow about forty pages on the somatic and behavioral aspects of phylogenetic and ontogenetic development of vertebrates. Next are seven chapters on the senses, two on reflexes, postures, and cerebral motor functions, one on emotion, one on sleep and activity, three on instinctive behavior and bodily needs, three on learning, one on aphasia, and one on symbolic processes. There are 176 well chosen illustrations and an alphabetized bibliography of 830 titles.

Those seeking an introductory textbook in this field are likely to find their expectations amply fulfilled with respect to scope and extensity. Possibly, however, too much has been attempted for one volume of 622 pages, for certain of the chapters are less informative than corresponding chapters in current textbooks in psychology and biology regularly used by college students of sophomore and junior grade. Less than one fifth of the 26 chapters are pitched at a sufficiently advanced level to meet major needs of graduate students in psychology, physiology, and medicine.

In general, the style is lucid, but here and there it suffers from loosely constructed or ambiguous sentences. For example, when speaking of the

brain, Dr. Morgan says, "Impulses come to it and leave it to a considerable extent through the cranial nerves, yet sensory impulses from all of the body except the head, as well as the control of the actions of the body by the brain, must be carried out through the spinal cord" (p. 75). In respect to locomotion he says, " . . . appendages for locomotion first appear in such amphybia as frogs and toads and become most highly developed in birds and certain of the lower mammals" (p. 104). Although by prior passage his statement is restricted to vertebrates, one cannot but wonder what his thoughts were in respect to the appendages of fishes.

The author has a penchant for ill-considered generalizations, such as: "The chimpanzee, for example, can use knives, forks, spoons, and other utensils, almost as well as can man" (p. 104); " . . . it makes little difference which of several different animals' nervous systems we use for illustration, for all vertebrate animals show the same general features" (p. 70); " . . . we may make the general statement that little or no improvement is to be observed in visual mechanisms in the phyletic series" (p. 106); " . . . smell is of very little use to man" (p. 146); "Mammals are uniformly capable of learning mazes. Moreover, there are no significant differences, it appears, in the rate at which different animals learn such problems" (p. 109). The data supporting these inordinate generalizations are unimpressive, either as to completeness or as to evidence of their conclusive nature.

But still more significant than faults of the foregoing types are "off-hand" descriptions, omissions in listing, and misstatements in noncontroversial areas. For example, "Cranial ganglia are to be found here and there in the recesses of the skull near the several small holes in the skull which serve as entrances and exits for nerve fibers" (p. 73); also, the choroid coat is said to "surround" the eye (p. 162). In listing the main parts of the cerebral hemispheres he omits the corpus striatum (p. 77) and elsewhere says, "Corticopetal fibers, on the other hand, take their origin, almost without exception, in the nuclei of the thalamus" (p. 81). The following sentence is more confusing than enlightening. "By means of the Sylvian aqueduct which passes through the midbrain, separating it into a floor and a roof, the third ventricle is connected with the fourth ventricle contained within the caudal pons and rostral medulla. This narrows down caudally to a small canal which runs the length of the spinal cord in the gray commissure" (p. 101). The anterior lobe of the hypophysis is said to be devoid of nerve fibers and in figure 32 is so illustrated. This is not in accord with recent researches.

Although some of the chapters are less burdened with loose phraseology and erroneous statements than those from which the foregoing illustrations were taken, it is fair to say that all are in need of editorial revision and some of them should be thoroughly screened for factual errors and distortions of meaning. Widespread inaccuracies militate against the usefulness of the book for undergraduates in psychology and graduate students in psychology, physiology, or medicine. Stated briefly, it is the opinion of the reviewer that this book exemplifies premature publication of a work of considerable promise.

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